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Mechanism of the Hemolytic Anemia Induced by Nitrofurantoin (Furadantin®). Further Observations on the Incidence and Significance of "Primaquine-Sensitive" Red Cells. EXALL L. KIMBRO, JR., MARIE V. SACHS AND JOHN V. TORBERT 245

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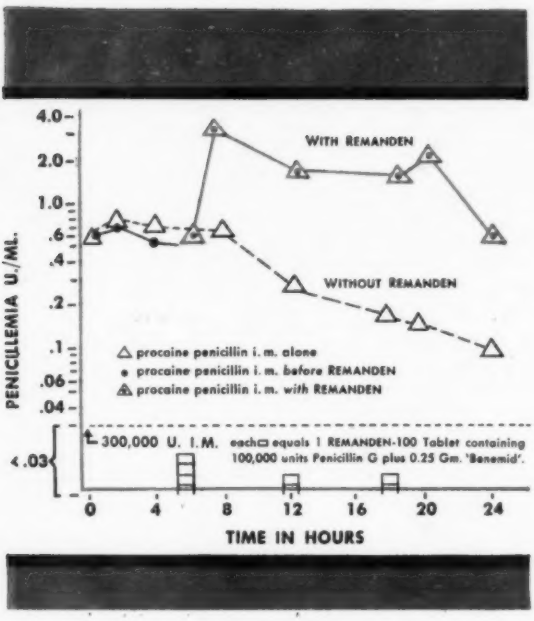

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MECHANISM OF THE HEMOLYTIC ANEMIA INDUCED BY NITROFURANTOIN (FURADANTIN®)[1]

Further Observations on the Incidence and Significance of "Primaquine-Sensitive" Red Cells

EXALL L. KIMBRO, Jr., MARIE V. SACHS and JOHN V. TORBERT[2]

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Received for publication July 2, 1957

Hemolytic anemia was observed to develop in two Negro males during the administration of nitrofurantoin (Furadantin®). The studies to be described indicate that hemolytic reactions to this drug may be limited to those individuals who have inherited a peculiar defect of the erythrocyte. This intrinsic abnormality of the red cells has been extensively studied by Dern and his associates (1–8) and has been shown to be responsible for the increased susceptibility of certain individuals to the hemolytic action of primaquine and a number of other drugs including sulfanilamide and acetanilid. The defect of the erythrocytes cannot be detected by usual hematologic examinations and appears to produce no untoward effects until the red cells are exposed to the offending chemical agent. The presence of the abnormality can be recognized by demonstrating that the concentration of reduced glutathione (GSH) in the red cells decreases when blood is incubated *in vitro* with acetylphenylhydrazine (9). The GSH of normal erythrocytes is stable under the same test conditions.

CASE REPORTS

Case. 1 E. F. (JHH # 462282), a 42 year old Negro male was admitted to The Johns Hopkins Hospital on October 24, 1955, because of congestive heart failure. There was no family history of anemia or drug reaction. The patient was known to have had inadequately treated syphilis with the subsequent development of aortic insufficiency and recurrent episodes of cardiac decompensation. These episodes were each associated with transient hepatomegaly and bilirubinemia. Hematocrit values were repeatedly normal. On the present admission, physical examination revealed a chronically ill man in slight respiratory distress. The sclerae were mildly icteric. The lungs were congested, the heart markedly enlarged, and the legs edematous. There was moderate hepatomegaly, but no splenomegaly. Hematocrit value was 44 per cent, white cell count 6,800 with normal differential count and the red cells appeared normal except for a few target cells. Total serum bilirubin was 4.4 mgm. per 100 ml.

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[1] Presented in part before the American Society for Pharmacology and Experimental Therapeutics, Federation Proceedings, Chicago, Ill., April 18, 1957.

[2] Clinical Fellow of The American Cancer Society.

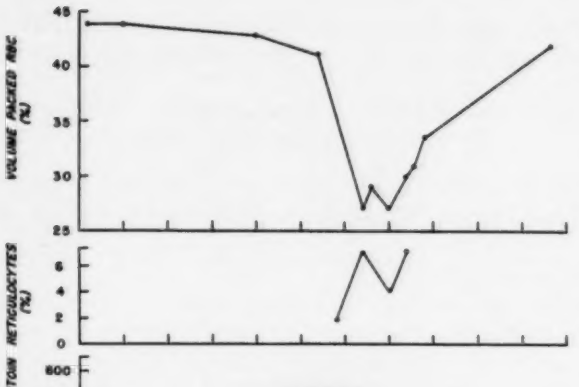


FIG. 1. Effect of the administration of nitrofurantoin on hematocrit values and reticulocytes in Case 1.

of which 3.0 mgm. were direct reacting. Initial medications included digitoxin, aminophyllin, sulfisoxazole (Gantrisin®) for two days only, and oxytetracycline (Terramycin®) for one day. The signs of decompensation gradually disappeared, and the hematocrit value remained normal. Because of persistent pyuria, nitrofurantoin, 400 mgm. per day was begun on November 16th. Four days later scleral icterus was pronounced. At this time the hematocrit value was 41 per cent, and the urine was clear yellow. Liver function studies the following day revealed a total serum bilirubin of 5.7 mgm. per 100 ml. with 2.8 mgm. direct reacting, thymol turbidity 3.6 units, cephalin flocculation negative, alkaline phosphatase 3.1 Bodansky units and BSP retention 1.9 mgm. per 100 ml. Reticulocyte count was 1.9 per cent, and the direct Coombs' test negative. Electrophoresis of the hemoglobin showed only hemoglobin A. There was no hepatosplenomegaly. Nine days after institution of nitrofurantoin therapy the hematocrit value had fallen to 27 per cent, and the reticulocyte count was 6.5 per cent (Fig. 1). The drug was discontinued. Two stool specimens were guaiac negative. Twelve days after nitrofurantoin was first given, the red cell count was 2.64 million per cmm., hemoglobin 7.9 gm. per 100 ml., hematocrit value 27.2 per cent, icterus index 8 units, MCV 103 cμ, MCH 30 $\mu\mu$g, MCHC 29 per cent, reticulocytes 4.1 per cent, platelets 260,000 per cmm., WBC 6,300 with normal differential count and on the blood smear there were a few target cells with moderate anisocytosis and polychromatophilia. Thereafter, the hematocrit value rose steadily and was 42 per cent one month after institution of nitrofurantoin therapy.

Case 2. S.G. (JHH # 641438), a 48 year old Negro male, was admitted to The Johns Hopkins Hospital on September 18, 1956, because of subarachnoid hemorrhage. There was no family history of anemia or drug reaction. His previous health had been good except for moderately severe hypertension, and he had had a cerebrovascular accident in 1953. On the day of the present admission, he developed left hemiparesis and lapsed into semi-coma. On admission he was found to have slight cardiomegaly, and the blood pressure was 196/130 mm. Hg. Hematocrit value was 45 per cent, icterus index less than 10 units, white cell count

9,550 with normal differential count and the red cells appeared normal on the blood smear. The initial medications included hydralazine (Apresoline®) for twenty-four hours, penicillin and streptomycin. One week after admission, the patient began to improve rapidly, and his sensorium cleared. Nine days after admission he developed an acute urinary tract infection for which nitrofurantoin, 400 mgm. per day, was prescribed. Penicillin was discontinued. The hematocrit value was 39 per cent, icterus index less than 10 units and white cell count 17,200. The patient showed marked improvement within three days. On the fourth day following institution of nitrofurantoin therapy, he again became acutely ill with fever, jaundice, tachycardia and somnolence. There was no hepatosplenomegaly, and urinalysis revealed only persistent pyuria. Two blood cultures were sterile. At this time the hematocrit value was 29 per cent, icterus index 18 units, and white cell count 13,800. Examinations of the stool for occult blood, performed daily during nitrofurantoin therapy, gave negative results. The direct Coombs' test was negative. The hemoglobin was normal on electrophoresis. Nitrofurantoin and streptomycin were discontinued and tetracycline (Achromycin®) therapy was instituted. The hematologic data pertinent to the patient's course are presented in Figure 2. Five days after nitrofurantoin was first given, liver function studies revealed a total serum bilirubin of 2.2 mgm. per 100 ml. with 1.2 mgm. in the indirect fraction, thymol turbidity 1.8 units, cephalin flocculation negative, and alkaline phosphatase 3.9 Bodansky units. The hematocrit value had dropped to 25.5 per cent with a reticulocyte count of 2.4 per cent. Transfusion of 1,000 ml. of whole blood was given. The bilirubinemia subsided, but slight reticulocytosis persisted. Thirty-five days after the first dose of nitrofurantoin the hematocrit value was 37.5 per cent, icterus index 7.5 units, reticulocyte count 0.6 per cent, white cell count 4,250, and total bilirubin less than 0.8 mgm. per 100 ml. To study the hemolytic reaction, nitrofurantoin, 400 mgm. per day, was re-administered and continued for ten days. There followed in six days a moderate fall in hematocrit value and subsequently a slight reticulocytosis. The hematocrit value then rose spontaneously within three weeks to a stable level of 40 per cent. Nitrofurantoin was once again administered in daily doses

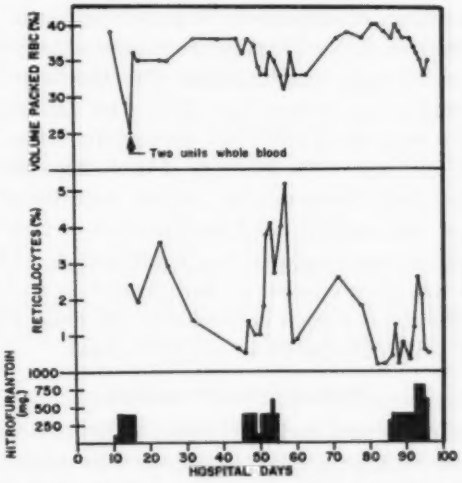


FIG. 2. Effect of the administration of nitrofurantoin on hematocrit values and reticulocytes in Case 2.

of 400 mgm. per day, seventy-five days after the patient's initial dose of the medication. The pre-administration blood values were: red cell count 3.79 million per cmm., hemoglobin 11.1 gm. per 100 ml., hematocrit value 39.1 per cent, icterus index 5.0 units, reticulocyte count 0.2 per cent, and white cell count 5,300 with normal differential count. Red cells and platelets appeared normal on the smear. Osmotic and mechanical fragility tests performed on fresh and incubated blood specimens gave normal results. On the eighth day of drug administration, the hematocrit value had fallen to 36.8 per cent and the fecal urobilinogen was elevated to 585 Ehrlich units per 100 gm. of feces. The daily dose of nitrofurantoin was then increased to 800 mgm. per day for four more days. There was a further drop in hematocrit value to 33.5 per cent by the eleventh day of administration and at this time fecal urobilinogen was 500 Ehrlich units per 100 gm. of feces.

MATERIALS AND METHODS

Glutathione determinations

The reduced glutathione (GSH) content of heparinized fresh whole blood samples (0.2 mgm. sodium heparin per milliliter of blood) was determined according to the method of Grunert and Phillips, as modified by Beutler (9). The optical density of the GSH-nitroprusside complex was measured at a wavelength of 520 millimicrons in a spectrophotometer.

GSH stability to acetylphenylhydrazine

Thoroughly oxygenated, heparinized, fresh whole blood was incubated with acetylphenylhydrazine and the reduced glutathione content subsequently measured as described by Beutler (9).

GSH stability to nitrofurantoin

Appropriate quantities of suspensions of nitrofurantoin in absolute methyl alcohol were pipetted into 125 x 15 mm. test tubes to prepare tubes containing 0.001, 0.01, 0.1, and 0.5 mgm. nitrofurantoin. The tubes were dried in a drying oven at a temperature not greater than 85°C. One milligram quantities of nitrofurantoin were weighed directly into appropriate tubes. The tubes were stored in the dark until used. One milliliter of fresh, heparinized, whole blood was added to a test tube containing the desired quantity of nitrofurantoin.[3] A glass stirring rod was used to aid the suspension of nitrofurantoin in the blood, and the mixture was incubated in a water bath at 37°C. At the end of one hour, the tube was removed from the bath, stirred for about thirty seconds, and replaced. At the end of a total of two hours, the blood was removed from the bath, and the GSH determined as described above.

Heinz body determinations

The appearance of red cell inclusion bodies ("Heinz bodies") *in vitro* on incubation of blood with acetylphenylhydrazine was studied by the method

[3] The concentrations of nitrofurantoin employed *in vitro* span the probable *in vivo* serum concentrations.

of Beutler, Dern and Alving (6). By substituting buffered solutions of nitrofurantoin for the acetylphenylhydrazine buffer solution in the standard test procedure, the formation of Heinz bodies by nitrofurantoin was studied. Two concentrations of nitrofurantoin were used, one containing 0.02 mgm. nitrofurantoin per ml., and the other containing 0.20 mgm. nitrofurantoin per ml.

Osmotic and mechanical fragility tests

Osmotic fragility tests on samples of defibrinated blood were performed according to the method described by Shen, Ham and Fleming (10). The optical density of the oxyhemoglobin solutions was read in a spectrophotometer (Beckman, Model B) at a wave-length of 543 millimicrons. The pH of the blood was adjusted within a range of 7.2–7.6 by mixture of the blood with carbon dioxide.

Mechanical fragility tests were performed by the method of Shen, Castle and Fleming (11). The hematocrit value of 1.0 ml. of defibrinated blood was adjusted to approximately 35 per cent. One-half milliliter of this sample was rotated at 30 r.p.m. for one hour at 37°C. in a 50 ml. Erlenmeyer flask containing ten 4 mm. glass beads. The optical density of the oxyhemoglobin solutions was determined as described above.

Separate samples of sterile defibrinated blood were incubated for two hours and for twenty-four hours at 37°C., with and without the addition of 1.0 mgm. nitrofurantoin per ml., and the osmotic and mechanical fragility of the erythrocytes subsequently determined by the methods described above.

Hemoglobin electrophoresis

Filter paper electrophoresis of hemoglobin was performed according to the method described by Smith and Conley (12).

Fecal urobilinogen

Fecal urobilinogen was estimated by the technique of Watson, Schwartz Sborov and Bertie (13).

RESULTS

A. Effect of incubating the patient's red cells with acetylphenylhydrazine

The reduced glutathione (GSH) content of the erythrocytes of the patient (S.G.) was determined before and after incubation with acetylphenylhydrazine. These results were compared with the results obtained by measurement of the GSH content of blood samples from four Negro individuals who had previously been found to have the red cell abnormality described by Dern and his associates (1), and also with the results obtained by similar measurements made on blood samples from four normal individuals (Fig. 3). There was some overlap in the values obtained from normal and abnormal cells prior to incubation.

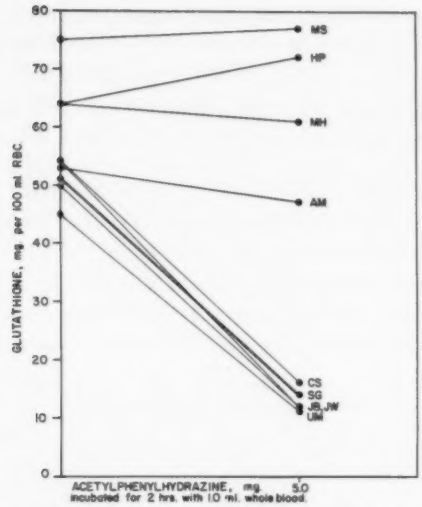


FIG. 3. Reduced glutathione concentration of blood before and after incubation with acetylphenylhydrazine. M. S., H. P., M. H. and A.M. are normal individuals. C. S., J. B., J. W. and U. M. are individuals known to have red cells of the primaquine-sensitive type. S.G. is the patient in Case 2.

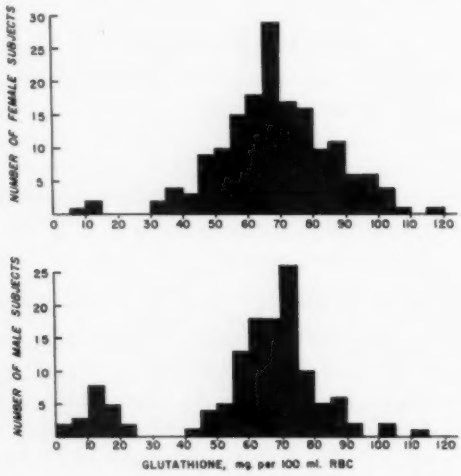
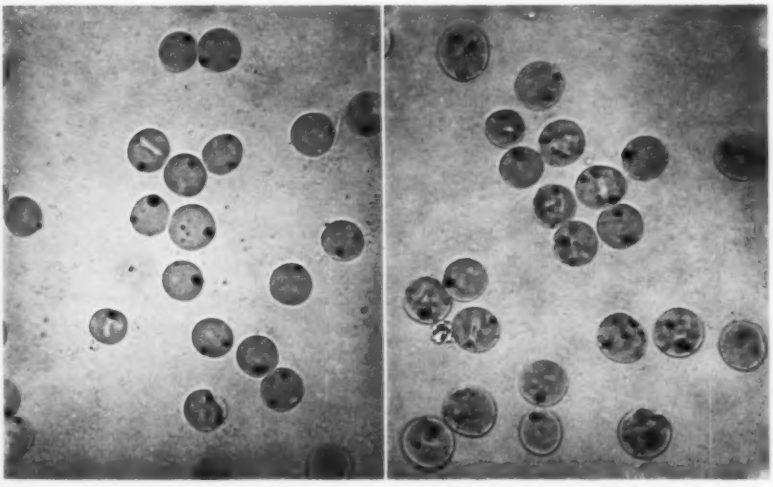


FIG. 4. Reduced glutathione concentration after incubation with acetylphenylhydrazine of blood obtained from 296 Negroes.

However, following incubation with acetylphenylhydrazine, the GSH content of the normal cells changed little, whereas the GSH content of the patient's cells and of the known abnormal cells fell precipitously. This observation demonstrated that the patient had the intrinsic red cell abnormality described by Dern and his associates (1).

This glutathione stability test was applied to blood samples obtained from 296 Negroes from the clinic population. The results, shown in Figure 4, demonstrated that in the group of males there were no GSH values between 22 and 40 mgm. per 100 ml. In the group of 165 females, 3 subjects had GSH values of 15 mgm. per 100 ml. or less. All but 6 of the remaining 162 individuals had GSH values greater than 40 mgm. per 100 ml. Six female subjects had GSH values between 30 and 40 mgm. per 100 ml. For the purpose of designation in this report, all subjects whose erythrocyte GSH fell on incubation with acetylphenylhydrazine to a level of 22 mgm. per 100 ml. or less will be referred to as "reactors," and are presumably susceptible to the hemolytic effect of primaquine and certain other drugs.

Heinz body formation during incubation of blood with acetylphenylhydrazine was measured in blood from the patient and from a normal individual. There was a marked increase in Heinz body formation in the patient's erythrocytes, as compared with the number formed in normal red cells (Fig. 5).



A B

FIG. 5. Heinz bodies in the red cells of a normal person (A) and of the patient in Case 2 (B) after incubation of the blood with acetylphenylhydrazine (× 790).

B. Effect of incubating the patient's blood with nitrofurantoin

GSH was measured in blood samples from the patient (S.G.) and from each of the other subjects referred to in Figure 3. One milliliter aliquots of whole blood from these same samples were incubated with 0.001, 0.01, 0.1, 0.5, and 1.0 mgm. quantities of nitrofurantoin for two hours at 37°C. A striking fall was produced in the GSH content of both the patient's and the "reactors'" blood by as little as 0.1 mgm. of nitrofurantoin per ml., whereas there was little change in the GSH content of the normal cells following their incubation with as much as 1.0 mgm. per ml. (Fig. 6).

Heinz bodies were not observed in the patient's erythrocytes during the administration of nitrofurantoin, and Heinz body formation *in vitro* could not be demonstrated in erythrocytes of the patient or in those of a normal individual following incubation of the cells with nitrofurantoin.

Figure 7 shows the results of measuring the GSH content of blood samples from 128 adult subjects after incubating separate aliquots of each sample with acetylphenylhydrazine and nitrofurantoin respectively. The 128 subjects included 3 Caucasians and 125 Negroes, of whom 19 were "reactors." It is apparent that the GSH content after incubation with nitrofurantoin was approximately the same as that following incubation with acetylphenylhydrazine, using the concentrations of each agent as specified.

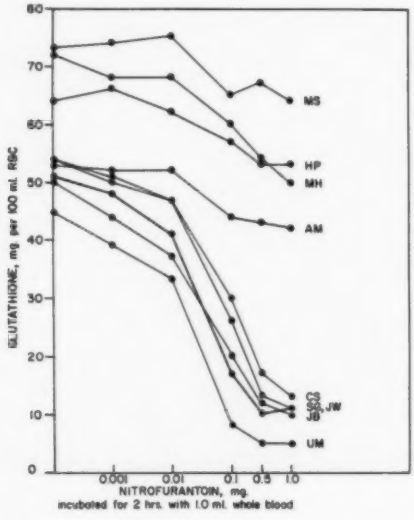


FIG. 6. Reduced glutathione concentration of blood before and after incubation with several concentrations of nitrofurantoin. M. S., H. P., M. H. and A. M. are normal individuals. C. S., J. W., J. B. and U. M. are individuals known to have red cells of the primaquine-sensitive type. S. G. is the patient in Case 2.

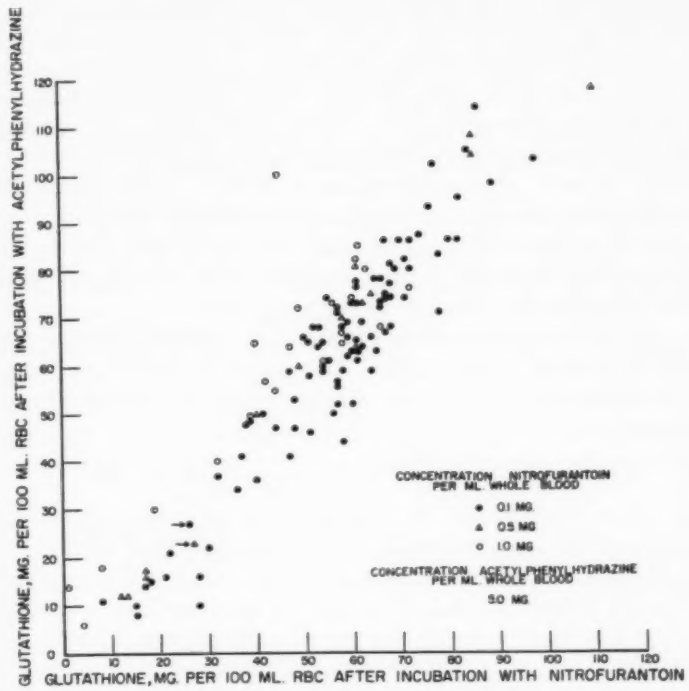

GLUTATHIONE, MG. PER 100 ML. RBC AFTER INCUBATION WITH ACETYLPHENYLHYDRAZINE
GLUTATHIONE, MG. PER 100 ML. RBC AFTER INCUBATION WITH NITROFURANTOIN
CONCENTRATION NITROFURANTOIN PER ML. WHOLE BLOOD
0.1 MG.
0.5 MG.
1.0 MG.
CONCENTRATION ACETYLPHENYLHYDRAZINE PER ML. WHOLE BLOOD
5.0 MG.

FIG. 7. Comparison of the reduced glutathione concentration of blood samples from 128 adult subjects after incubating separate aliquots of each sample with 5 mgm. acetylphenylhydrazine per ml. whole blood and with various concentrations of nitrofurantoin respectively. The results indicated by the arrows designate determinations made on samples obtained from "reactors" immediately following acute hemolytic episodes.

C. Erythrocyte fragility studies

Osmotic and mechanical fragility tests on the erythrocytes of the patient (S.G.) performed before and after incubation at 37°C. for twenty-four hours were normal. Incubation of 1.0 mgm. of nitrofurantoin with 1.0 ml. of sterile, defibrinated blood for twenty-four hours at 37°C. did not increase the osmotic or mechanical fragility of erythrocytes of "reactors" and hemolysis did not occur *in vitro*.

D. Stability of GSH in other cases of hemolytic anemia

Six patients who had previously experienced acute, self-limited hemolytic episodes were demonstrated to be "reactors" on the basis of GSH instability of their blood. Clinically, it had been thought likely that the episodes were drug-induced. One hemolytic episode followed the concurrent ingestion of 666,® a proprietary preparation containing antipyrine, ammonium chloride,

citric acid and magnesium sulfate, and Anacin,® which contains phenacetin, acetylsalicylic acid and caffeine. Another episode followed administration of phenacetin to a patient who had not developed anemia during long term para-aminosalicylic acid and isoniazid therapy. One episode followed the administration of probenecid (Benemid®) to a patient on long term paraaminosalicylic acid and streptomycin therapy; this patient had received a nine-day course of sulfadiazine in 1941 without developing hemolytic anemia. Two patients suffered hemolytic episodes after ingestion of naphthalene and their cases have been reported by Zinkham and Childs (14). The sixth patient had acute hemolytic anemia which is thought to have been induced by an unidentified medication that the patient had taken just prior to admission.

The GSH of blood from a white patient with Gantrisin®-induced pancytopenia and from Negro patients with homozygous hemoglobin S disease and homozygous hemoglobin C disease was stable on incubation with acetylphenylhydrazine.

E. Incidence of the red cell abnormality in Negroes

Blood from a large group of Negroes was incubated with acetylphenylhydrazine and the GSH measured. Figure 4 shows the results of this study. In a random group of 296 Negro employees, out-patients, and in-patients of The Johns Hopkins Hospital, a total of 23 "reactors" was found, an incidence of 7.8 per cent. It is of interest that 165 of the 296 subjects were females, but that only 3 of the 23 "reactors" were in the female group, an incidence of 1.8 per cent. Twenty "reactors" were found among the 131 Negro males, an incidence of 15.3 per cent.

DISCUSSION

On the basis of the observations described, it seems probable that nitrofurantoin may induce hemolytic anemia in the same subjects who would be expected to have a hemolytic reaction during administration of primaquine, and by the same or a closely related mechanism. In the only previously reported case of hemolytic anemia attributed to nitrofurantoin, the patient was a Negro male (15), as were both of our patients. Similarity is apparent in the clinical courses of the three patients, except for the absence of hemoglobinemia and hemoglobinuria in the two subjects of this report.

One of the patients was challenged with nitrofurantoin on two occasions soon after the initial hemolytic episode. Each time a definite but less marked hemolytic response was induced. Progressive lessening of the hemolytic response to drug administration is also seen in primaquine-induced hemolytic anemias (2), and has been shown to be due to the fact that only the older cells in the circulation are susceptible to the noxious effect of the drug (4).

Although the drug-sensitive erythrocytes are apparently lysed *in vivo* by

the action of primaquine (1) or nitrofurantoin, one interesting and as yet unexplained difference between the effects of the two agents on the abnormal cells is their respective abilities to induce formation of Heinz bodies. Following administration of primaquine to a sensitive subject, or incubation of sensitive red cells with primaquine, Heinz bodies are found in a large percentage of the cells (3, 6). Under the same circumstances, nitrofurantoin did not induce Heinz body formation.

The nature of the inherited abnormality of the erythrocyte which underlies the unique susceptibility to hemolysis remains to be established. Our studies agree with Beutler's conclusions that these drug-sensitive cells are normal in respect to morphology, hemoglobin type, osmotic and mechanical fragility, and that the direct Coombs' test is negative; further, there is no hemolysis *in vitro* and no increase in the osmotic or mechanical fragility following incubation of the red cells with the offending drugs (3). Carson and his associates have demonstrated a deficiency of glucose-6-phosphate dehydrogenase activity of primaquine-sensitive erythrocytes (16), and it is possible that deficiency of this enzyme is the fundamental defect.

In the group of 296 Negroes whose red cell GSH stability to acetylphenylhydrazine was tested, none of the non-sensitive males had GSH values less than 40 mgm. per 100 ml.; however, 6 of the 162 females not included in the "reactor" group had GSH values between 30 and 40 mgm. per 100 ml. This is of particular interest, since the incidence of "reactors" in the female group was noted to be less than that in the male group. The results of studies clarifying the nature of this relationship, and also illustrating the characteristics of the genetic transmission of the erythrocyte abnormality will be reported (17).

It has become apparent that hemolysis of the abnormal red cells can be produced by many agents. Zinkham and Childs have shown that hemolytic anemia caused by ingestion of naphthalene occurs principally in individuals with this erythrocyte abnormality (14). They have also found that the red cell GSH of newborns is almost uniformly unstable to acetylphenylhydrazine, and it is thought that the susceptibility to hemolysis by vitamin K substitutes in the newborn period may be related to this GSH instability (18).

SUMMARY

The development of acute hemolytic anemia in two Negro patients following administration of nitrofurantoin (Furadantin®) has been described. Re-administration of nitrofurantoin induced further hemolysis in one of these patients and *in vitro* studies revealed the red cell GSH to be unstable on incubation with acetylphenylhydrazine, an abnormality common to erythrocytes uniquely susceptible to hemolysis by primaquine and certain other drugs. The GSH of primaquine-sensitive red cells was shown to be unstable also to incubation with small amounts of nitrofurantoin, whereas normal erythrocytes maintained their

glutathione in the reduced state following incubation with high concentrations of nitrofurantoin. It seems probable that nitrofurantoin may induce hemolytic anemias in the same subjects who could be expected to have a hemolytic reaction during administration of primaquine, and by the same or a closely related mechanism.

Random screening of 296 Negroes revealed the incidence of the intrinsic red cell abnormality to be 7.8 per cent, with predominant occurrence in males. Clinical observations suggest that this abnormality may be the basis for numerous instances of drug-induced hemolytic anemia.

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AN IMMUNOHISTOLOGIC STUDY ON THE OCCURRENCE OF INTRAVASCULAR ANTIGEN-ANTIBODY PRECIPITATION AND ITS ROLE IN ANAPHYLAXIS IN THE RABBIT[1]

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The present study was undertaken to determine the nature and role of amorphous "thrombi" observed in small pulmonary vessels of rabbits that have been subjected to anaphylactic shock.

The signs of anaphylactic shock differ markedly from one species to another. Because of their great susceptibility, guinea pigs have been most widely employed in the study of acute anaphylaxis. In this species, death from anaphylactic shock is caused by respiratory failure resulting from constriction of the bronchioles due to contraction of the bronchiolar musculature and, to a lesser extent, peribronchial edema. On the other hand, anaphylactic shock in the rabbit is characterized by tachypnea, nystagmus, weaving of the head, and prostration. These signs are thought to be the result, either directly or indirectly, of marked obstruction in the pulmonary capillary bed as evidenced by the increased pressure required to perfuse fluid through the pulmonary artery, by dilatation of the right side of the heart, and by peripheral circulatory collapse.

In addition to these different manifestations of sudden antigen-antibody interaction, there are also marked differences in the quantitative immunologic aspects of anaphylactic shock in the guinea pig and rabbit (1). Kabat and Landow have shown that the intravenous injection of 30 micrograms of antibody nitrogen is sufficient to produce uniformly fatal anaphylactic shock in guinea pigs challenged with antigen 48 hours later (2). On the other hand, Jackson found that high levels of *circulating* antibody, of the order of 500 micrograms antibody nitrogen per ml. of serum, were required to elicit severe anaphylactic shock in the rabbit (3). Even this large amount of circulating antibody did not ensure fatal anaphylaxis.

The obstruction of the pulmonary circulation in the rabbit undergoing anaphylactic shock is usually attributed to spasm of the pulmonary vascular bed. Although numerous experiments have been carried out to determine the

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mechanism of shock, no extensive histologic studies have been done. Vaubel found pulmonary capillary "thrombi" in three rabbits dying of anaphylactic shock following several intravenous injections of horse serum (4). In rabbits which perished a short time after the last of multiple serum injections, Sato described distention of the pulmonary capillaries by amorphous masses having the appearance of plasma or fibrin (5). In rabbits subjected to several injections of horse serum, Gregory and Rich described capillary "thrombi" as a frequent component of pulmonary lesions which they considered comparable to rheumatic and anaphylactic pneumonitis in man (6). Most of the aforementioned investigators believed that the amorphous intracapillary masses were "thrombi" resulting from focal capillary damage produced by the antigen-antibody reaction.

While this study was under way, our attention was directed to a report by Dixon on the use of I^{131} labeled antigens in immunologic investigation, in which he described concentrations of radioactive antigen in eosinophilic masses occluding the pulmonary capillaries of several rabbits subjected to anaphylactic shock. He thought it likely that the intracapillary "thrombi" were intravascular I^{131} antigen-antibody precipitates (7).

During previous studies on experimental "serum sickness," widespread hyaline "thrombi" were often observed in the pulmonary capillaries and arterioles of rabbits receiving repeated injections of bovine albumin or bovine gamma globulin. When these rabbits showed signs of anaphylactic shock, pulmonary "thrombi" were invariably present (8, 9). On the other hand, pulmonary "thrombi" of this type were never encountered in rabbits developing "serum sickness" after a single injection of antigen under conditions where sudden, massive antigen-antibody interaction did not occur (10). Consideration of these findings led to the belief that the pulmonary "thrombi" might be the result of intravascular antigen-antibody precipitation. This supposition was tested by using fluorescein-labeled antigens.

The following abbreviations have been used:

BSA = Crystallized bovine serum albumin.
Fluor-BSA = Fluorescein-labeled BSA.
BGG = Bovine serum gamma globulin.
Fluor-BGG = Fluorescein-labeled BGG.
AbN = Antibody nitrogen.
AtN = Antigen nitrogen.

EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1: The Demonstration of Intravascular "Thrombi" in Passive Anaphylactic Shock in Rabbits.

Previous experiments had shown that the formation of intravascular amorphous masses in the lungs were invariably present in anaphylactic shock in

TABLE I

Amorphous Eosinophilic "Thrombi" in Pulmonary Capillaries Following Passive Anaphylactic Shock in the Rabbit

Rab. No.	Antibody* injected; mg. anti-BSA nitrogen	Interval between injection of antibody and antigen	Challenging injection of BSA, mg. nitrogen	Signs of anaphylactic shock†	Precipitates in capillaries of lung
698	44	0	16	None	+
660	60	30 min.	80	Rapid resp., weakness, nystagmus, weaving of head, convulsive movements. Recovered in 20 min.	+
663	60	48 hr.	80	Restlessness.	+
697	87	0	80	None	+
691	87	30 min.	80	Rapid resp., weakness, weaving of head, urination, defecation. Recovered in 30 min.	+
693	87	2 hr.	80	Weaving of head, defecation	+
661	90	30 min.	80	Weakness, collapse with head held to one side	+
664	90	48 hr.	80	Restlessness. Defecation.	+
692	174	30 min.	160	Rapid resp., weaving of head, weakness, defecation.	+

* Antiserum obtained from rabbits receiving weekly injection of 20 mg. crystallized bovine albumin in Freund's adjuvant for 4 months.

† Rabbit #692 sacrificed at 30 min.; all others sacrificed 1 hour after antigen injection.

rabbits which had undergone active sensitization. The present experiment was undertaken to determine whether intravascular "thrombi" would be observed in normal animals passively sensitized with antibody and challenged thereafter with antigen.

Potent antiserum was obtained from rabbits receiving weekly injections of 20 mg. crystallized bovine albumin (Armour and Co.) in Freund's adjuvant for a period of 4 to 5 months. The antiserum was calibrated for its antibody-N content by the method of Heidelberger and Kendall (11). Varying quantities of antiserum (antibody-N) were injected intravenously into rabbits, and the rabbits were challenged with BSA at intervals of time ranging from 0 to 48 hours.

As shown in Table I, most of the rabbits treated in the above fashion exhibited signs characteristic of anaphylactic shock in this species. However, even with amounts of antibody many times that sufficient to produce lethal shock in guinea pigs, death did not occur.

Most of the rabbits in the present experiment were sacrificed within 1 hour after the challenging injection of antigen. Hematoxylin and eosin sections of the lungs of all the rabbits revealed widespread eosinophilic amorphous "thrombi" plugging the capillaries and small arterioles.

Experiment 2: A Study on the Nature of the Intravascular "Thrombi" using Rabbits Sensitized to Bovine Albumin and Challenged with Fluorescein-labeled Bovine Albumin.

(a) Sensitization of animals.

A group of rabbits were immunized with alum-precipitated crystallized bovine serum albumin according to the method of Kabat and Mayer (11). Each rabbit received a total of 90 mg. BSA divided into twenty injections given during a period of 5 weeks. Thereafter, in order to produce very high antibody titers, the rabbits received 140 mg. BSA incorporated in Freund's adjuvant mixture, divided into 7 weekly subcutaneous injections. Approximately 1 week after the last injection of antigen, the animals were bled. One-half ml. quantities of serum from each rabbit were mixed with varying quantities of antigen contained in 0.5 ml. physiologic saline. After 24 hours in the cold, precipitates were removed by centrifugation, and the supernatant fluids were tested for excess antigen with a potent antiserum. Approximate equivalence values in terms of antigen could thus be determined for the serum of each rabbit. By assuming the ratio of antibody nitrogen to antigen nitrogen in the equivalence zone to be 5:1 for the bovine albumin-rabbit antibody system, the approximate antibody content for the sera could be calculated. Animals with approximately the same high antibody levels were chosen for use in the experiment.

(b) Preparation of fluorescein-labeled bovine serum albumin and bovine gamma globulin.

Fluorescein amine was coupled to crystallized bovine albumin and bovine gamma globulin according to the method of Coons and Kaplan (12). The conjugated material was dialyzed against buffered saline until the fluid outside the dialysis bag remained visibly free of greenish-yellow color.

The fluorescein-labeled antigens were almost completely precipitated from solution by the addition of potent pooled antiserum from rabbits which had been sensitized against the respective unlabeled proteins. The supernatant fluid after centrifugation of the precipitates showed only a trace of greenish-yellow color. The fluorescein-labeled solutions were calibrated by Kjeldahl nitrogen determination (11).

(c) Preparation of unlabeled antigen solutions.

Crystallized bovine serum albumin and bovine gamma globulin were dissolved in buffered saline in a concentration of 100 mg. per ml. (16 mg. of nitrogen).

(d) Injection of antigen solutions.

A total of 8 animals previously sensitized to BSA were employed. Four of these animals were challenged with fluorescein-labeled BSA; 4 others, as con-

trols, received a solution containing *unlabeled BSA* and an equal quantity of fluorescein-labeled BGG to determine whether the localization of the fluorescein-labeled BSA was specific. Two normal animals were injected with fluorescein-labeled BSA to determine the distribution of this antigen in the absence of antibody. All of the antigen solutions were injected in a volume of 20 ml. at a contsant rate during 1 minute.

(e) Preparation of tissues and examination by fluorescence microscopy.

After injection of the antigen solution, all animals were observed for signs of anaphylactic shock. Those which did not die within 10 minutes were killed with intravenous pentobarbital sodium. Immediately after death all animals were autopsied, and the tissues were fixed in 20 per cent formalin, embedded in paraffin, and sectioned.

Tissue sections were studied with a Leitz Ortholux microscope fitted with photomicrographic accessories and both tungsten and ultraviolet light sources. A dark field arrangement was devised by placing a 9 mm. black disc on the copper sulfate chamber below the substage condenser. This facilitated photographic reproduction and was more satisfactory than a cardioid condenser. Ultraviolet absorption filters mounted above the slide and in the oculars protected the microscope objectives, the photographic film, and the eye of the observer from ultraviolet irradiation. The bright yellow-green light emanating from the fluorescent antigens, the blue autofluorescence of elastic tissue, and the faint white autofluorescence of certain tissue cells were easily distinguishable.

Even in the hematoxylin and eosin stained sections, one would see the characteristic yellow-green fluorescence of the conjugated antigens when these sections were viewed with the ultraviolet light source, and, therefore, by switching from ultraviolet to ordinary tungsten illumination, one could determine the location of the antigens in the tissues. However, the stains and the mounting medium in these preparations emanated a disturbing additional fluorescence for black and white photography; therefore, the following procedure was used. Unstained glycerin-mounted sections were first photographed with ultraviolet illumination. Then the glycerin was removed, the section stained with hematoxylin and eosin, and the same microscopic field again photographed with ordinary illumination.

Photographic film with an exposure index of 200 was used, and 15 minutes exposure was required to record the fluorescence.

(f) Results.

The data are summarized in Table II. In hematoxylin and eosin preparations, amorphous eosinophilic "thrombi" were seen in profusion, obstructing the pulmonary capillaries of sensitized rabbits challenged with fluorescein-

TABLE II

The Demonstration of In Vivo Antigen-Antibody Precipitation in BSA Sensitized Rabbits Using Fluorescent BSA as Challenging Antigen

Rab. No.	Circulating antibody level; mg. anti-BSA N per ml.	Challenging Antigen			Amorphous Precipitates in capillaries of lungs (H & E stain)	Fluorescence of precipitates in capillaries of lungs (unstained)	Signs of anaphylatic shock
		Fluor-BSA, mg. of N	Solution of:				
			Unconjugated BSA mg. of N	Fluor-BGG; mg. of N			
Rabbits sensitized to BSA:							
225	2	6.4			+	+	0
141	1.6	6.4			+	+	+
222	1	16			+	+	+
286	1.6	16			+	+	+
217	1.6		6.4	6.4	+	0	+
213	2		6.4	6.4	+	0	0
224	2		16	16	+	0	+ Died, 7 min.*
218	1.6		16	16	+	0	+ Died, 4 min.*
Normal Rabbits:							
157	0	6.4			0	0	0
158	0	6.4			0	0	0

* Remaining rabbits sacrified 10 minutes after antigen injection.

labeled BSA. In many instances the capillaries were enormously distended by these "thrombi." When examined by ultraviolet light, the "thrombi" gave a brilliant yellow-green fluorescence, and with this light source it was possible to see many smaller intravascular masses which were not appreciated when the hematoxylin and eosin sections were first examined. In the other organs these "thrombi" were fewer in number and in H & E sections difficult to identify. However, in the glomeruli of the kidneys, in the liver, and in the spleen, where many vascular channels are readily seen, such "thrombi" were common. In the liver they were sometimes quite large, filling the terminal portal veins and extending into the sinusoids. In the tiny capillaries of the brain it was almost impossible to identify the hyaline "thrombi" in the stained sections, but under ultraviolet light the yellow-green color readily distinguished them from red cells or plasma packed in a capillary.

In control animals sensitized to BSA but receiving *unlabeled* BSA mixed with fluorescein-labeled BGG, similar eosinophilic hyaline and granular intracapillary "thrombi" were seen in H & E sections. However, when viewed with ultraviolet light these "thrombi" were not fluorescent, although some contained minute particles which did show a yellow-green fluorescence. Whether these particles represented antibody fixation of fluorescein-labeled BSA impurities in the fluorescein-labeled BGG or whether they were bits of fluorescent BGG caught up mechanically in the precipitating masses could not be determined.

It was strikingly apparent, however, that no significant amount of fluorescent heterologous antigen had become included in the "thrombi."

In normal unsensitized control rabbits which received fluorescein-labeled BSA no capillary "thrombi" were found. Under ultraviolet light only faint tinges of yellow-green were seen in a few patches of plasma within large vessels. The cytoplasm of macrophages in the lungs, spleen, and liver showed variable amounts of yellow-green fluorescence.

Experiment 3; The Demonstration of *in vivo* Antigen-Antibody Precipitation in Rabbits Sensitized to Bovine Gamma Globulin and Challenged with Fluorescein-labeled Bovine Gamma Globulin.

A group of rabbits were given 7 weekly subcutaneous injections of 20 mg. of bovine gamma globulin in Freund's adjuvant mixture. Approximate antibody levels were determined, as described in Experiment 2, assuming the antibody-antigen weight ratio for the BGG-antibody system to be 3.5 to 1. The techniques employed were the same as those described with BSA; four animals sensitized to BGG were challenged with fluorescein-labeled BGG. Four other BGG sensitized animals, serving as controls, were challenged with a solution of *unlabeled* BGG and fluorescein-labeled BSA. Two normal rabbits received fluorescein-labeled BGG. The results are recorded in Table III.

In rabbits sensitized to BGG and challenged with fluorescent BGG, H & E

TABLE III

The Demonstration of Intravascular Antigen-Antibody Precipitation in the Lungs of BGG Sensitized Rabbits following Shock with Fluorescent BGG

Rab. No.	Approx. circulating antibody: mg. anti-BGG N per ml.	Challenging Antigen			Amorphous precipitates in pulmonary capillaries (H & E stain)	Fluorescence of precipitates (unstained sections)	Signs of anaphylactic shock
		Fluor-BGG, mg. of N	Solution of				
			Unconjugated BGG, mg. of N	Fluor-BSA, mg. of N			
Rabbits sensitized to BGG:							
153	0.7	6.4			+	+	+
136	0.7	6.4			+	+	+
152	0.7	16			+	+	+
155	0.7	5.3			+	+	+ Died, 2 min.*
140	0.7		6.4	6.4	+	0	+
151	0.7		6.4	6.4	+	0	+
137	0.7		16	16	+	0	+
138	0.7		16	16	+	0	+ Died, 3 min.*
Normal Rabbits:							
157	0	6.4			0	0	0
158	0	6.4			0	0	0

* Remaining rabbits sacrificed at 10 minutes.

sections showed large eosinophilic "thrombi" in the capillaries of the lungs and in the other organs listed. Under ultraviolet light, bright yellow-green fluorescence was sharply localized to these amorphous masses. In control animals sensitized to BGG, but injected with *unlabeled* BGG mixed with fluorescein-labeled BSA, intracapillary "thrombi," localized predominantly in the lungs, were seen in the H & E sections, but these did not show fluorescence under ultraviolet illumination. In the normal unsensitized animals which received fluorescein-labeled BGG, no intracapillary masses were observed, and the only localized yellow-green fluorescence seen was in the fixed macrophages of the spleen and liver. The observations in this experiment were entirely similar to those in Experiment 2 where the BSA system was employed.

Experiment 4: Studies on the Role of Antigen-Antibody Precipitation in the Production of Anaphylactic Shock in the Rabbit.

(a) The effect of intravenous washed immune precipitates prepared *in vitro.*

Precipitates were made by the addition of fluorescein-labeled bovine albumin to rabbit antiserum, the resultant mixture containing excess antibody. After incubation at 37°C. for 30 minutes, the precipitates were collected by centrifugation, twice washed, and resuspended in cold saline.

As shown in Table IV, 3 normal rabbits injected intravenously with washed immune precipitates, contained in 15 ml. of saline, showed signs of severe shock indistinguishable from those observed in actively and passively sensitized rabbits. Sections of lungs from these three animals showed fluorescent intracapillary masses, similar to those occurring when antigen was injected into highly sensitized rabbits. These animals were not sacrificed until 30 hours after injection, and there was, by this time, conspicuous leucocytic infiltration in and about the intravascular precipitates.

TABLE IV

The Results in Normal Rabbits of the Intravenous Injection of Washed Immune Precipitates Prepared with Fluorescent BSA and Excess Anti-BSA Rabbit Antibody

Rab. No.	Fluor-BSA, Mg. N contained in precipitate	Signs of "anaphylactic shock"*	Intravascular amorphous precipitation in lungs (H & E stain)	Fluorescence of precipitates
102	5.6	Severe weakness, nystagmus, weaving of head, marked tachypnea, prostration.	+++	+
103	2.8	Weakness, nystagmus, weaving of head, tachypnea.	++	+
104	1.4	Weakness, nystagmus, weaving of head, slight tachypnea.	+	+

* All rabbits sacrificed 30 hours after antigen injection.

(b) The effect of soluble antigen-antibody complexes (prepared in antigen excess) as compared with antigen-antibody precipitates (prepared in antibody excess).

(1) Non-fluorescent immune precipitates were prepared by the addition of 20 mg. BSA nitrogen (1.25 ml.) to each of 4 tubes containing 100 ml. of anti-BSA rabbit serum, the antibody content of which was approximately 1 mg. AbN per ml. After 20 minutes at room temperature, the tubes were centrifuged and the precipitate thoroughly washed with 100 ml. of cold saline. After additional centrifugation, the precipitate in each tube was taken up in 60 ml. of cold saline and finely suspended by homogenization in a Waring blendor. The resulting suspension readily passed through a 22 gauge needle. Kjeldahl analysis of the suspension showed 2.0 mg. antigen-antibody N per ml. Three rabbits were injected intravenously with 48 ml. of this suspension. All three showed tachypnea, weakness, and weaving of the head, but the signs of shock were not as severe as in the preceding three animals, perhaps because the particle size in the precipitate suspension was smaller as a result of homogenization. The rabbits were sacrificed within one hour. Hematoxylin and eosin preparations of the lungs showed eosinophilic amorphous masses in the capillaries.

(2) In a previous study, it was demonstrated that typical anaphylactic shock could be obtained in normal guinea pigs by the injection of soluble antigen-antibody complexes (13). Since the present study suggested that *in vivo* antigen-antibody precipitation may be responsible for most of the signs encountered in anaphylactic shock in the rabbit, it was considered of importance to establish the effect of *soluble* antigen-antibody complexes in this species. Accordingly, to each of 20 tubes containing 10 ml. of anti-BSA rabbit serum (2 mg. AbN per ml.) was added 2 ml. BSA (8 mg. N per ml.). This great excess of antigen completely prevented precipitation. Forty-eight ml. of this solution (equivalent to 80 mg. AbN and 64 mg. AtN presumably in the form of soluble antigen-antibody complexes and free antigen) were injected intravenously into each of 3 rabbits. It should be pointed out that each rabbit given precipitates in part *1* received approximately 77 mg. AbN and 19 mg. of BSA-N. Except for slight tachypnea, soluble complexes produced no effect, and, as was expected, sections showed no precipitates in the pulmonary capillaries. To test the effectiveness of these soluble complexes, each of 6 guinea pigs was injected with 2 ml. of this solution and, of these, 3 died in typical anaphylactic shock, 1 exhibited a slight reaction, and 2 gave no response.

Experiment 5: Focal Necrosis in the Liver Following Injection of Antigen and Immune Precipitates into the Mesenteric Veins of Sensitized and Normal Rabbits Respectively.

TABLE V

The Effect of Antigen in Sensitized Rabbits and Immune Precipitates in Normal Rabbits when Injected Via the Portal Circulation

Rab. No.	Sensitizing antigen	Approx. mg. AbN per ml. serum	Material injected	Time between injection and death, hrs.*	Precipitates in intrahepatic portal veins and sinusoids	Fluorescence of precipitates	Periportal and midzonal necrosis of liver
128	BSA	.7	Fluor-BSA, 4.2 mg. N	24 (S)	+	+	+
129	BSA	.7	Fluor-BSA, 5.4 mg. N	4.5 (D)	+	+	+
130	BSA	.7	BSA, 4.8 mg. N and Fluor-BGG, 3.2 mg. N	0.5 (D)	+	−	−
150	BGG	.4	Fluor-BGG, 4.8 mg. N	24 (S)	+	+	+
154	BGG	.4	Fluor-BGG, 4.8 mg. N	24 (D)	+	+	+
156	BGG	.4	Fluor-BGG, 4.8 mg. N	0.5 (S)	+	+	−
100	Normal	—	Washed immune ppt. containing BSA, 4.8 mg. N, + excess antibody	24 (S)	+	−	+
101	Normal	—		24 (S)	+	−	+

* D = Died; S = Sacrificed.

The occurrence of precipitates in the terminal portal veins and hepatic sinusoids of rabbits undergoing anaphylactic shock in the preceding experiments suggested a possible explanation for the old and puzzling observation of "focal allergic necrosis of the liver" following repeated injections of foreign protein (14, 15, 16, 17). Accordingly, rabbits previously sensitized to bovine albumin or bovine gamma globulin were challenged by the injection of fluorescein-labeled antigens directly into the portal circulation. In addition, normal animals were injected by the same route with washed immune precipitates. All animals were shaved and anesthetized with pentobarbital sodium and ether. Under sterile conditions, a loop of large bowel was delivered through a midline abdominal incision, and the antigen or immune precipitate was injected into a small mesenteric vein.

The procedural data and results are outlined in Table V. In hematoxylin and eosin sections of the livers from animals sensitized to BSA and challenged with fluorescein-labeled BSA, typical eosinophilic masses were seen in the terminal portal veins and in adjacent sinusoids, and, as was expected, they showed bright yellow-green fluorescence under the ultraviolet microscope. In the control animal sensitized to BSA and receiving unlabeled BSA mixed with fluorescein-labeled BGG, no fluorescence was observed in the precipitates except for occasional tiny particles similar to those seen in control animals in the previous experiments. There was fluorescence of the cytoplasm of some Kupfer cells. No parenchymal alterations were present in animals killed or

dying within 4½ hours after injection, but in animals surviving for 24 hours many, often fairly large, portal and midzonal hepatic necroses were seen. At this time, the precipitates and the necrotic areas were heavily infiltrated with polymorphonuclear leucocytes. When the precipitates in the portal end of the sinusoids and the adjacent necrotic foci lay in the same plane of the section, a most convincing visual impression was obtained suggesting that obstruction of the vascular channels by precipitates had led to infarction of the liver cells.

In animals sensitized to BGG and challenged with fluorescein-labeled BGG, the results were entirely similar to those described for the BSA system. In the normal unsensitized animals, sacrificed 24 hours after receiving precipitates made *in vitro*, there were amorphous intravascular masses identical in appearance and distribution to those seen in the sensitized animals. Focal areas of necrosis were also observed in the portal zones of these animals.

DISCUSSION

The present study indicates that intravascular amorphous "thrombi," occurring predominantly in the pulmonary circulation, are characteristic of acute anaphylactic shock in the rabbit. When the challenging antigen was labeled with fluorescein amine, it was possible to demonstrate specific localization of antigen in the "thrombi." The observations strongly suggest that the intravascular "thrombi" are antigen-antibody precipitates, since the "thrombi" were fluorescent only when the challenging fluorescein-labeled antigen was the antigen to which the animal had been sensitized.

In most studies concerned with the mechanism of anaphylactic shock in the rabbit, the conclusion has been drawn that this condition results from spasmodic contraction of the pulmonary blood vessels (18, 19, 20). Drinker and Bronfenbrenner many years ago remarked on the similarity of acute anaphylactic shock in the rabbit to acute pulmonary embolism produced by foreign particulate matter (21). The present observations suggest that anaphylactic shock in the rabbit, where relatively huge quantities of circulating antibody are required, may be, to a large degree, the result of antigen-antibody precipitates forming in the circulation and then lodging in the small vessels of the lung, thereby causing vascular obstruction. In humans the lethal effects of multiple small pulmonary emboli of all sorts are well known but, to our knowledge, in none of these situations does the extent or severity of the embolization approach that seen in rabbits subjected to anaphylactic shock. Furthermore, it is of interest that normal rabbits injected with immune precipitates prepared *in vitro* may exhibit signs and anatomic changes indistinguishable from those seen in ordinary anaphylactic shock in sensitized rabbits. Whether *in vivo* immune precipitation is wholly responsible for anaphylactic shock in the rabbit is not clear. Large quantities of soluble antigen-antibody complexes capable of

eliciting anaphylactic shock in guinea pigs produced no effect in the rabbits tested. However, the possibility cannot be ignored that factors other than vascular obstruction by antigen-antibody precipitation may be important in the pathogenesis of anaphylactic shock in the rabbit.

In several sensitized animals challenged with fluorescein-labeled antigen and then sacrificed 12 to 120 hours later, there was marked leucocytic infiltration in and about the pulmonary capillary "thrombi" and extending into the alveolar sacs. Gregory and Rich described a similar form of focal pneumonitis in animals receiving multiple injections of horse serum and compared it to that observed in some human cases of rheumatic fever. They believed the leucocytic infiltration and thrombosis of the pulmonary capillaries were secondary to focal capillary damage. The present observations suggest that the experimental anaphylactic pneumonitis produced by these workers was the result of intravascular antigen-antibody precipitation brought about by a second or third injection of antigen into animals made hypersensitive by prior injections.

In the livers of sensitized animals receiving antigen and of normal animals receiving immune precipitates via the mesenteric vein, many "thrombi" were seen occluding the terminal branches of the portal vein and hepatic sinusoids. In animals killed more than 12 hours after such treatment, foci of necrosis in mid- and periportal zones of the hepatic lobule were common, presumably as a consequence of the obstruction to hepatic circulation. Similar necroses have been produced by injection of foreign particulate matter into the portal circulation (22).

In their study on "Experimental allergic focal necrosis of the liver," Hartly and Fushbough concluded that the local union of antigen and antibody was responsible for the focal hepatic necrosis and implied that this was a direct toxic effect on the liver cells and not secondary to ischemia, since only "occasional" thrombi were seen in portal veins and sinusoids, and that they were not common enough to account for the frequent necroses. In this regard, it should be pointed out that identification of antigen-antibody precipitates (or "thrombi") is uncertain in routine hematoxylin and eosin sections and that they are easily confused with pools of serum and even, on occasion, with clumps of red cells. It must be emphasized that not only in the lung, but also in the liver, following challenge via the mesenteric vein, the large numbers of immune precipitates can only be appreciated when sections are examined under the ultraviolet microscope following challenge with a fluorescein-labeled antigen. Under these conditions the frequent observation of precipitates occluding the portal veins and sinusoids entering a necrotic area is rather convincing evidence that ischemia and infarction are the reasons for the hepatic necrosis seen under the conditions of these experiments.

SUMMARY

1. Large amounts of antigen and antibody are required for the induction of anaphylactic shock in rabbits. *In vivo* intravascular precipitation of antigen with antibody, as demonstrated by the use of fluorescein-labeled antigen, regularly occurs under these conditions.

2. Obstruction of the pulmonary capillaries by immune precipitates appears to be an important mechanism in the pathogenesis of anaphylactic shock in the rabbit.

3. Obstruction of the portal veins and sinusoids of the liver by immune precipitates may cause infarction of the portal and midzonal areas of the liver lobule, especially when the challenging antigen is given via the portal circulation.

4. The foregoing facts suggest an explanation for the unusual aspects of anaphylaxis in the rabbit and must be considered when comparisons are made with other animals or man where *in vivo* antigen-antibody precipitation has not been demonstrated.

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EXPLANATION OF ILLUSTRATIONS

FIG. 1. Rabbit 222. Sensitized to bovine albumin, challenged with fluorescein-labeled bovine albumin. (a) Lung showing amorphous, eosinophilic intracapillary "thrombi." H & E. 300 ×. (b) Same microscopic field under ultraviolet illumination showing fluorescence (white foci) in "thrombi."

FIG. 2. Rabbit 224 (sensitized control for rabbit 222). Sensitized to bovine albumin, challenged with unlabeled bovine albumin and fluorescein-labeled bovine gamma globulin. (a) Lung showing amorphous, eosinophilic intracapillary "thrombi." H & E. 300. × (b) Same field under ultraviolet light showing absence of fluorescence in the "thrombi."

FIG. 3. Rabbit 155. Sensitized to bovine gamma globulin, challenged with fluorescein-labeled bovine gamma globulin. (a) Lung showing intracapillary "thrombi." H & E. 300 ×. (b) Same field under ultraviolet light showing fluorescence in "thrombi."

FIG. 4. Rabbit 138 (sensitized control for rabbit 155). Sensitized to bovine gamma globulin, challenged with unlabeled bovine gamma globulin and fluorescein-labeled bovine albumin. (a) Lung showing intracapillary "thrombi." H & E. 300 ×. (b) Same field under ultraviolet light showing absence of fluorescence in "thrombi."

FIG. 5. Rabbit 155 (same animal as in Fig. 3). (a) Brain showing questionable "thrombi" in minute capillaries. H & E. 300 ×. (b) Same field under ultraviolet light.

FIG. 6. Rabbit 222 (same animal as in Fig. 1). (a) Liver showing "thrombi" in portal vein with extension into sinusoids. H & E. 300 ×. (b) Same field showing fluorescence of "thrombi." Note that red blood cells in portal vein do not fluoresce.

FIG. 7. Rabbit 128. Sensitized to bovine albumin, challenged with fluorescein-labeled bovine albumin via the mesenteric vein; sacrificed 24 hours later. (a) Liver showing "thrombi" in small portal vein with surrounding focal necrosis and leucocytic infiltration. H & E. 170 ×. (b) Same field under ultraviolet light.

FIG. 8. Normal rabbit 101. Injected into the mesenteric vein with washed immune precipitates; sacrificed 24 hours later. "Thrombus" in small portal vein with necrosis and leucocytic infiltration of adjacent hepatic cells.

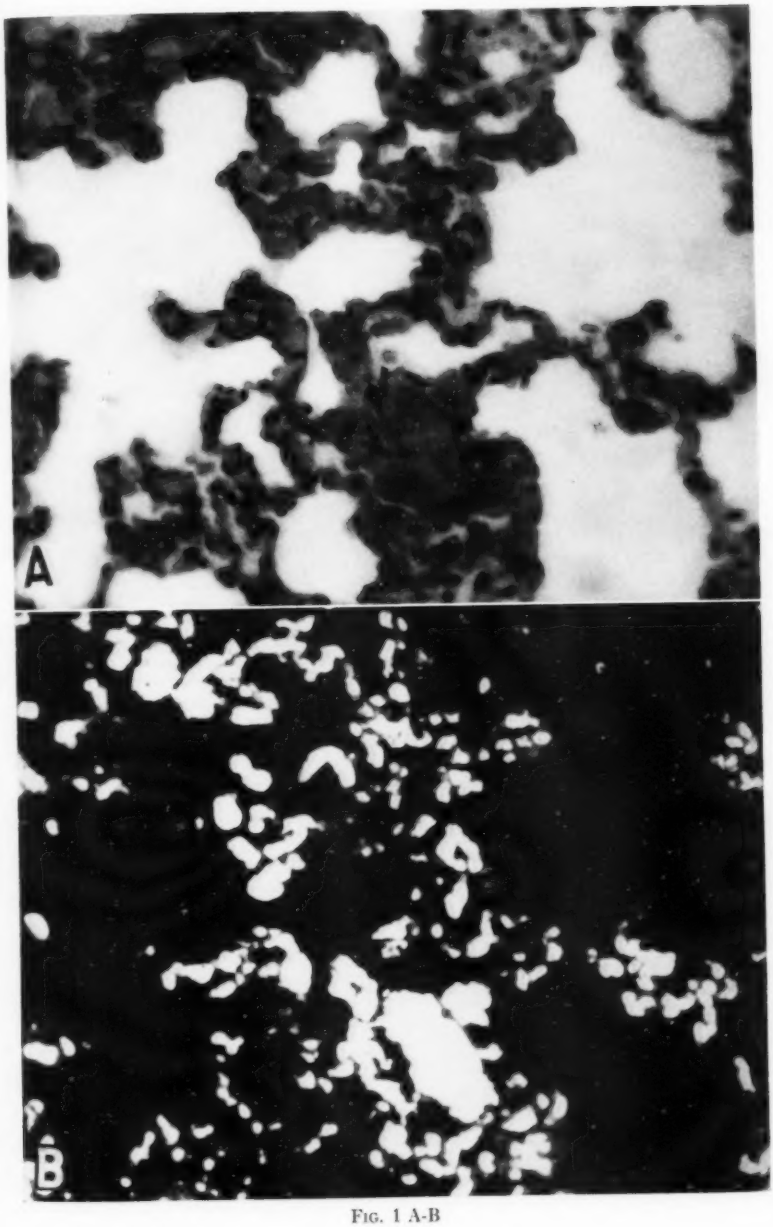

FIG. 1 A-B

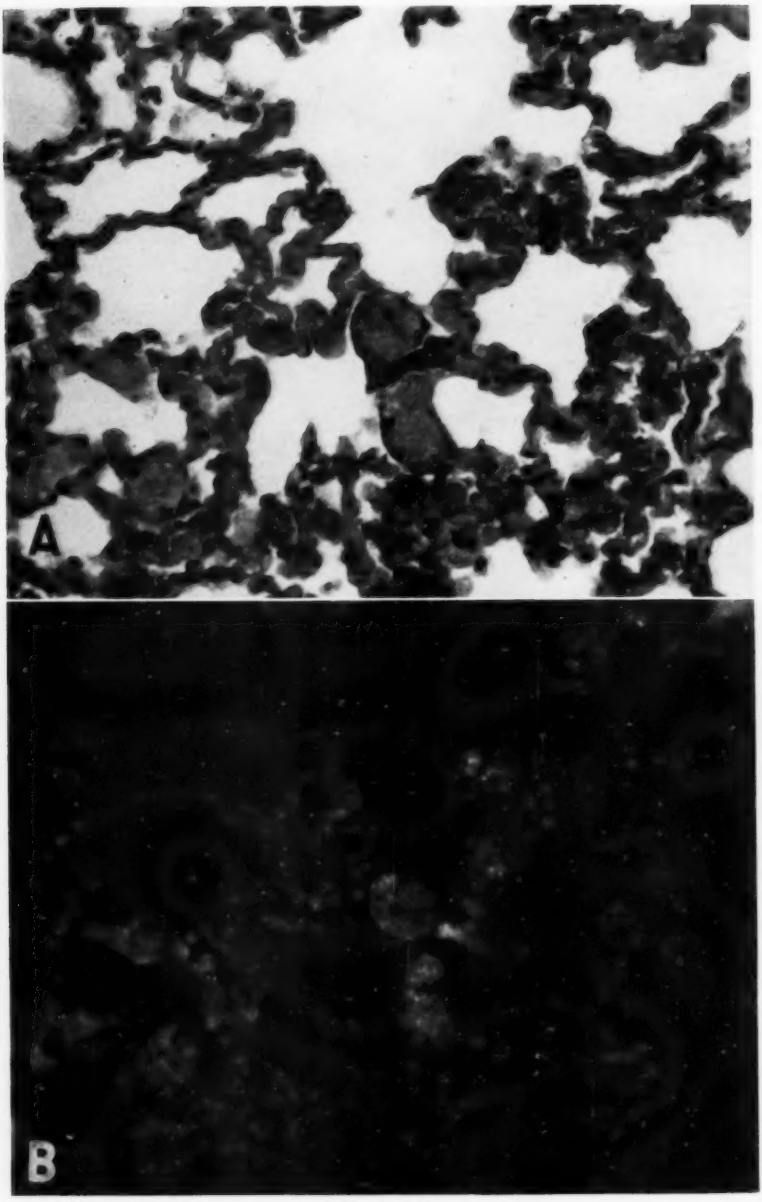

FIG. 2 A-B

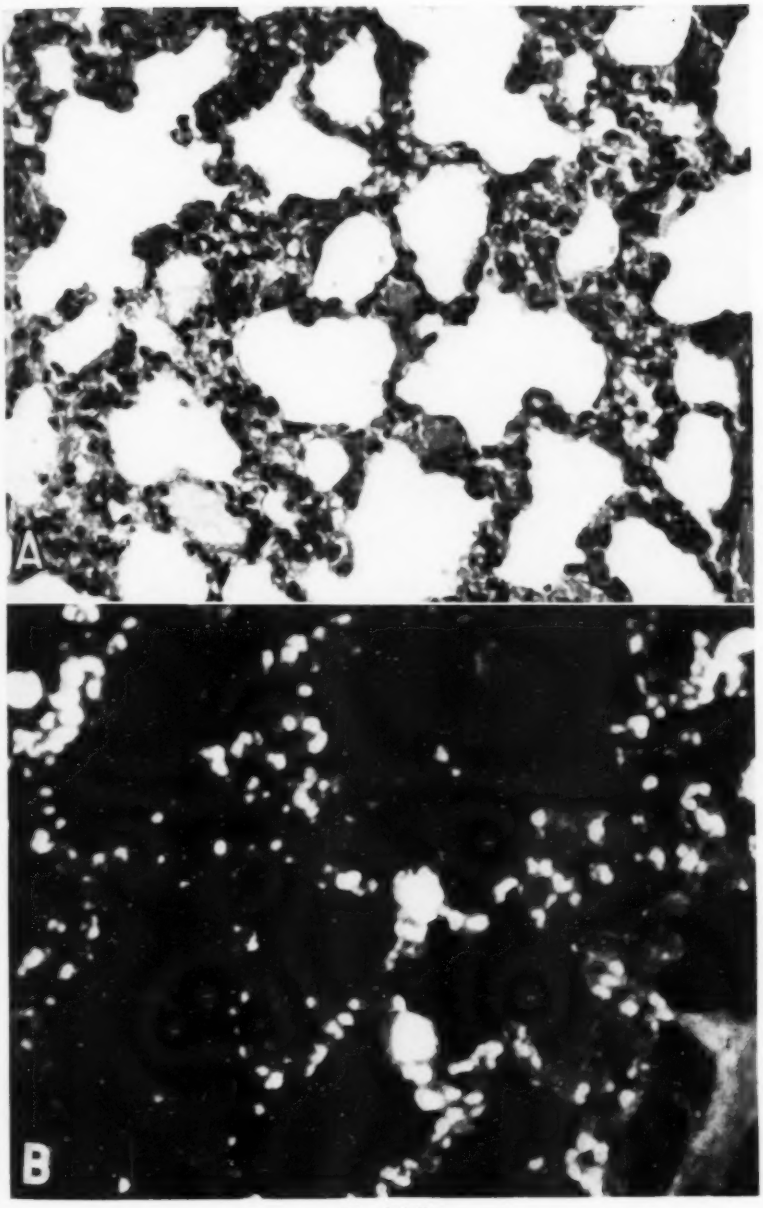

FIG. 3 A-B

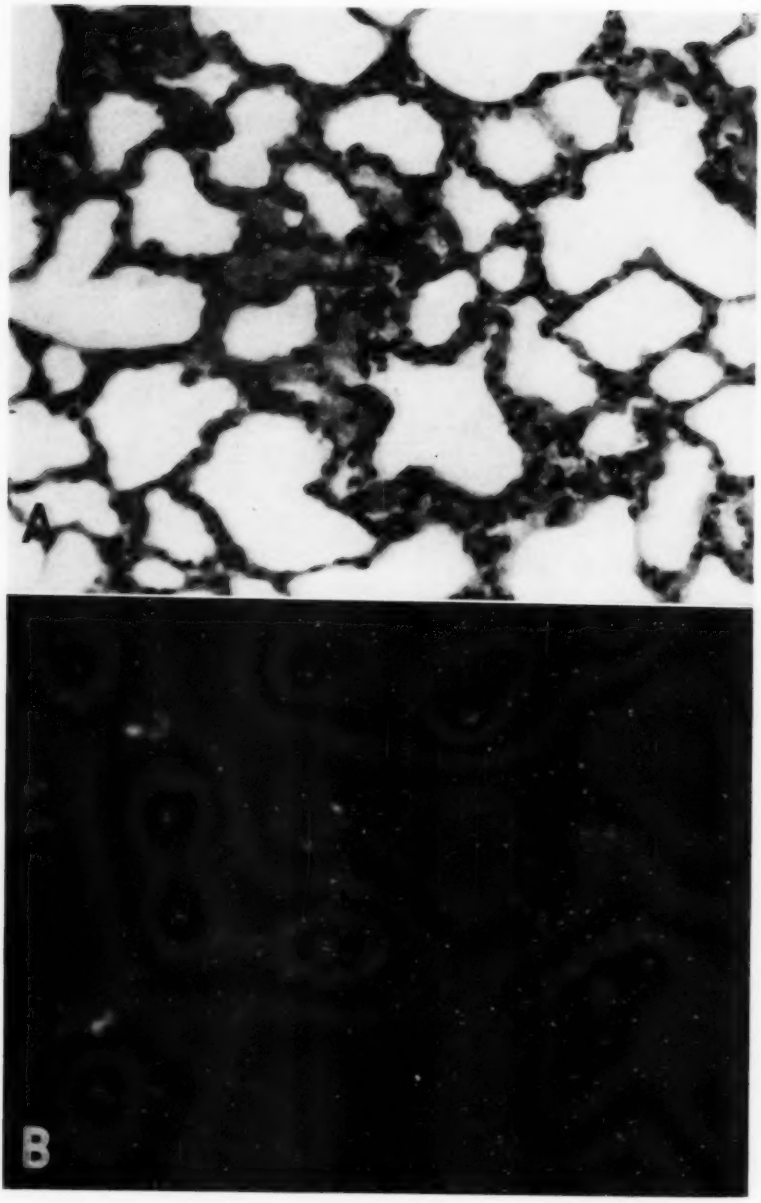

FIG. 4 A-B

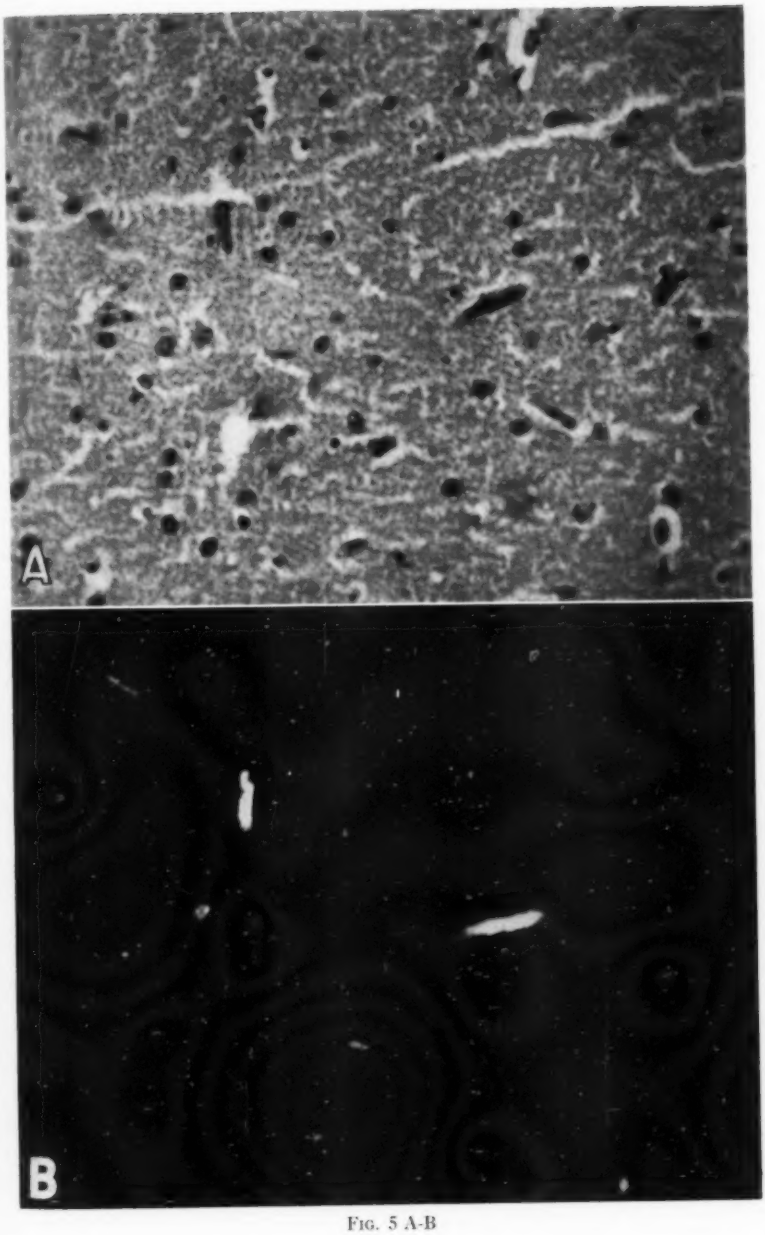

FIG. 5 A-B

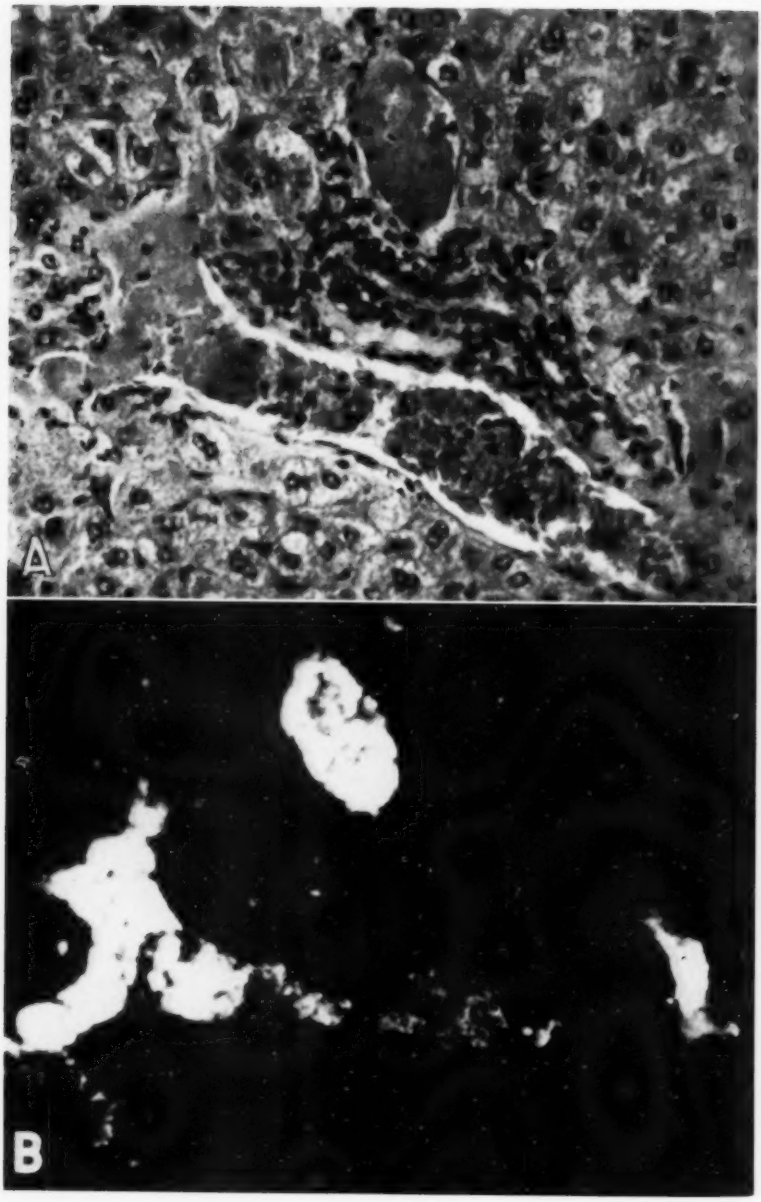

FIG. 6 A-B

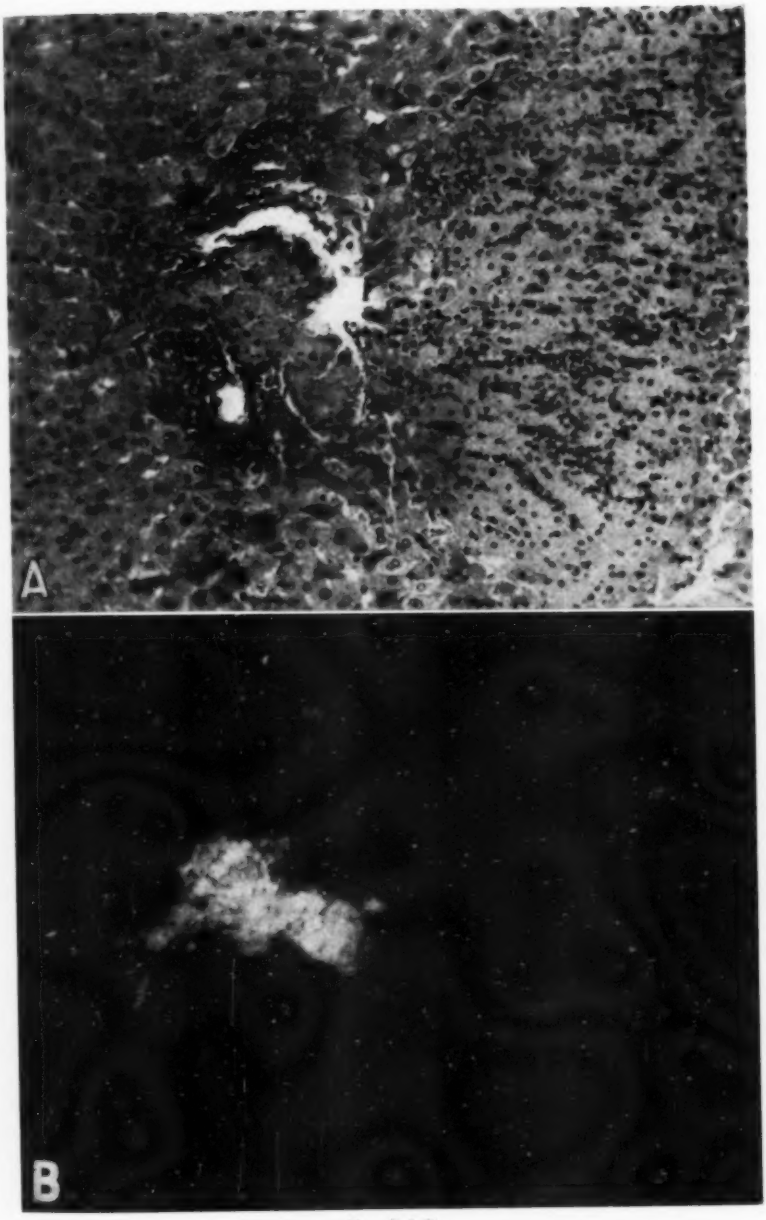

FIG. 7 A-B

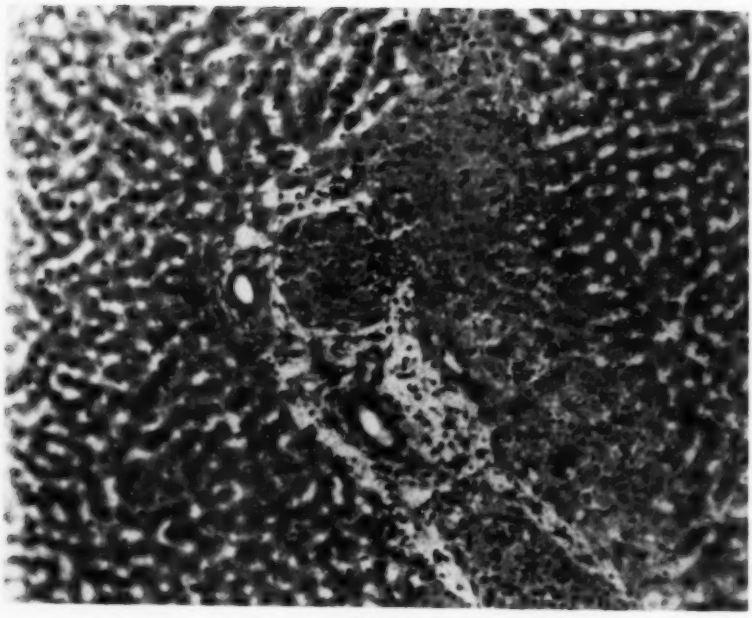

FIG. 8

FACTORS INFLUENCING THE ALTERATION OF THE PYROGENIC ACTION OF ENDOTOXIN BY SERUM[1]

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Addition of normal serum to bacterial endotoxin prior to inoculation into rabbits enhances the toxin's pyrogenic action (1–4). The febrile response of man to endotoxin is similarly augmented by whole blood (5). The effect of normal serum is visible as shortening of the lag period before onset and increase in height of fever.

Hegemann (6) has reported that incubation of endotoxin with normal serum at 37°C. causes a reduction in the toxin's pyrogenic action. This effect is demonstrable only when the period of contact of endotoxin and serum at 37°C. is prolonged for several hours prior to inoculation into *normal animals*. This inhibition is not seen after normal serum has been adsorbed with zymosan and is probably attributable to the properdin system.

These two contrasting effects of normal serum on endotoxin are dependent upon the length of time that serum and toxin are in contact *in vitro* at 37°C. before injection, as will be illustrated in this paper. Rabbits become tolerant to the action of endotoxin when given repeated daily injections. Serum from such animals does not augment fever (4.7) and this failure of tolerant rabbit serum to increase endotoxin's pyrogenic action has been investigated also.

MATERIALS AND METHODS

Endotoxins.—A purified protein-lipo-polysaccharide complex from *Shigella flexneri* was used (8). This toxin was dissolved in 0.85 per cent NaCl in a concentration of 1.0 mcgm./ml. and stored at 4°C. A purified endotoxin from *Serratia marcescens* (P-35), kindly supplied by Dr. M. J. Shear (National Cancer Institute, Bethesda), was used in a concentration of 10 mcgm./ml. and stored at 4°C.

Normal rabbit serum.—Normal male rabbits, of mixed breed and color, weighing 2.5 to 3.0 kg. were exsanguinated by cardiac puncture using aseptic precautions. The blood was allowed to clot in sterile, pyrogen-free 50 ml. centrifuge tubes at room temperature and the serum was pooled. An aliquot of the pooled serum was stored at −50°C. and the remainder, at 4°C.

Measurement of temperature.—The febrile response of rabbits was measured by an indwelling rectal resistance coil and a Telethermometer (4) every 30 minutes for 1 hour before intravenous injection of pyrogen and for 5 hours thereafter.

Tolerant rabbits.—Tolerant serum was obtained from rabbits given daily intravenous injections of 10 mcgm. of P-35 or 1.0 mcgm. of Shigella endotoxin for 3 weeks. Development

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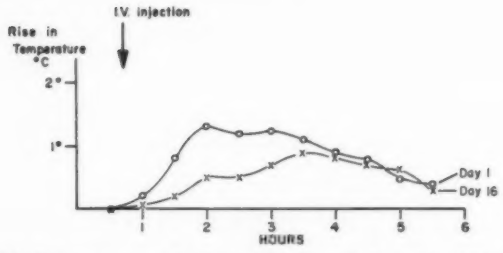

FIG. 1. Mean febrile response on the first and sixteenth day of 6 rabbits given daily intravenous injections of 1.0 mcgm. of Shigella endotoxin.

of tolerance was confirmed in every instance by recording the febrile response of animals after the first and last injections (*Fig. 1*). The day following the last inoculation each animal was bled by cardiac puncture; serum was pooled and stored as described for normal serum.

All studies on augmentation of fever were carried out in rabbits made tolerant to 1.0 mcgm. of Shigella toxin in 0.85 per cent NaCl by intravenous injection of this dose daily for 2 to 3 weeks. Tolerant rabbits were used because augmentation of fever by normal serum is more easily demonstrated in these than in normal animals. Febrile responses were recorded on the first and last day of injection of toxin-in-saline to confirm the development of tolerance in each animal. A different group of tolerant rabbits was used in each experiment.

Preparation of properdin deficient (RP) serum.—According to the Pillemer technique (9), 200 mg. of zymosan powder were suspended in 10 ml. 0.85 per cent NaCl and added to 100 ml. of serum. The mixture was placed in a water bath at 17°C. for 50 minutes, and centrifuged for 20 minutes at 3600 RPM in the cold. Zymosan extraction was carried out twice in this manner. Each pool of RP serum was shown to be free of contaminating bacterial pyrogen by injection of 4.0 ml. aliquots into normal rabbits and measurement of their temperature responses.

Properdin assays were kindly performed by Dr. Louis Pillemer (Western Reserve University, Cleveland).

EXPERIMENTAL RESULTS

A. *Factors influencing the effect of normal serum upon the febrile response to endotoxin.*—To determine the effect of varying the period of incubation of serum-endotoxin mixtures before injection, the following experiments were performed.

Two groups of 3 tolerant rabbits were studied. Each of the rabbits in one group was given an intravenous injection of 1.0 mcgm. of Shigella endotoxin which had been incubated with 4.0 ml. normal rabbit serum for 30 minutes at 37°C. The animals in the second group were injected intravenously with 1.0 mcgm. of toxin which had been incubated with 4.0 ml. of normal rabbit serum for 12 hours at 37°C. Febrile responses were recorded as described above. The following day the same experiment was repeated but the 2 groups of animals were reversed.

The mean febrile responses observed in these experiments are shown in

Figures 2 and 3. The fevers of individual animals corresponded closely to the means. There was marked augmentation of the febrile response to toxin incubated with normal serum for 30 minutes. In contrast, there was no enhancement of fever when incubation was carried out for 12 hours. These results indicate that the length of time serum and endotoxin are in contact at 37°C before injection into test animals is of crucial importance in determining the final effect upon body temperature.

To determine if enhancement of pyrogen fever by a short period of contact of normal serum with endotoxin is altered by treatment of serum with zymosan, and to confirm Hegemann's finding that RP serum failed to inhibit endotoxin fever, the following experiments were performed.

Two groups of 3 tolerant rabbits were studied. Each of the rabbits in one group was injected intravenously with 1.0 mcgm. of Shigella endotoxin that had been incubated with 4.0 ml. of RP serum at 37°C. for 30 minutes. The animals in the second group were given 1.0 mcgm. of toxin that had been incubated with 4.0 ml. of RP serum for 12 hours. The following day the experiment was repeated with the groups reversed. The properdin titer of the

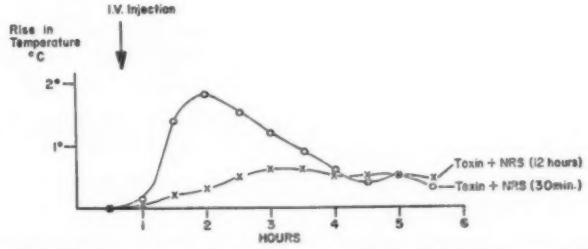

FIG. 2. Mean febrile response of 2 groups of 3 tolerant rabbits: One group inoculated with 1.0 mcgm. Shigella endotoxin which had been incubated for 30 minutes with 4.0 ml. of normal serum (T + NRS 30 min.). One group inoculated with 1.0 mcgm. of Shigella endotoxin which had been incubated for 12 hours with 4.0 ml. of normal rabbit serum (T + NRS 12 hours).

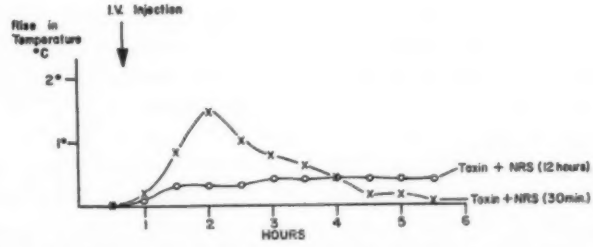

FIG. 3. Mean febrile response of the 2 groups of tolerant rabbits illustrated in Fig. 2. The same endotoxin-serum mixtures were injected but the group of animals were reversed.

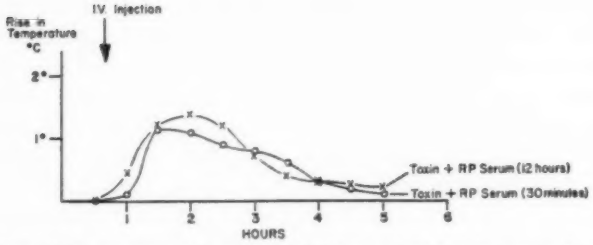

FIG. 4. Mean febrile response of 2 groups of 3 tolerant rabbits: One group inoculated with 1.0 mcgm. of Shigella endotoxin which had been incubated for 30 minutes with 4.0 ml. of zymosan-treated normal serum (RP-serum 30 minutes). One group inoculated with 1.0 mcgm. of Shigella endotoxin which had been incubated for 12 hours with 4.0 ml. of zymosan-treated normal serum (RP-serum 12 hours).

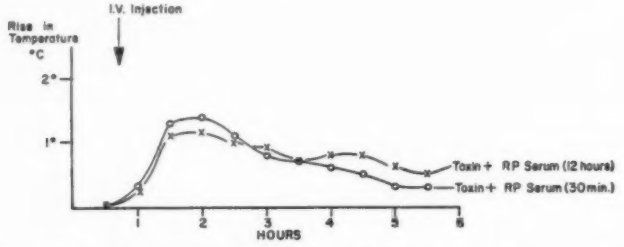

FIG. 5. Same experiment illustrated in Fig. 4 with the 2 groups of animals reversed.

serum used in these experiments was 12 units/ml. before and 1.0 unit/ml. after treatment with zymosan.

The mean fever curves obtained in these experiments are illustrated in *Figures 4 and 5*. Incubation with RP normal serum for 30 minutes or for 12 hours augmented the fever-producing action of endotoxin in all animals.

The heat lability of the properties of normal serum which affect pyrogen fever was studied in the following experiments.

Two groups of 3 tolerant rabbits were used. Each rabbit in one group was injected intravenously with 1.0 mcgm. of Shigella endotoxin that had been incubated at 37°C. for 12 hours with 4.0 ml. of normal rabbit serum which had been heated at 60°C. for 2 hours. Each of the animals in the other group was given 1.0 mcgm. of toxin which had been incubated at 37°C. for 12 hours with 4.0 ml. of normal serum.

There was no augmentation of the febrile response by unheated serum but heated serum enhanced endotoxin fever in all animals. Normal serum that had been stored at 4°C. for one month incubated with Shigella endotoxin at 37°C. for 30 minutes or for 12 hours also produced augmentation of febrile responses.

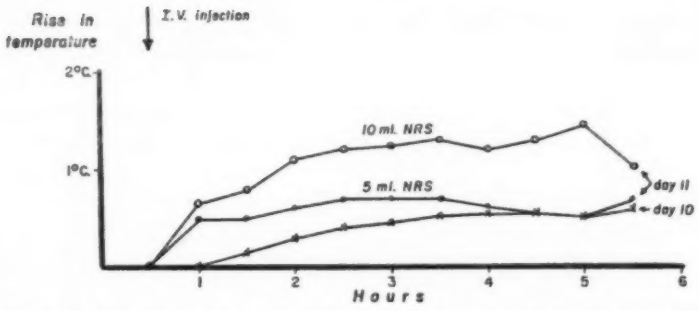

FIG. 6. The effect of increasing amounts of normal rabbit serum on the augmentation of endotoxin fever. Febrile responses of 12 rabbits on the tenth day of daily injection of 1.0 mcgm. of Shigella endotoxin-in-saline. Febrile response of the tolerant rabbits on day 11 to 1.0 mcgm. of toxin which had been incubated for 30 minutes with 5.0 or 10 ml. of normal serum.

The influence of increasing amounts of normal serum mixed with endotoxin in augmenting fever of tolerant rabbits was studied.

Three tolerant rabbits were injected intravenously with 1.0 mcgm. of Shigella endotoxin which had been incubated for 30 minutes with 5.0 ml. of normal serum. Another group of 3 tolerant animals received 1.0 mcgm. of endotoxin that had been incubated for 30 minutes with 10 ml. of normal serum. The augmentation of fever was greater in animals given 10 ml. of serum (*Fig. 6*). This suggests a stoichiometric relationship between the enhancing property of serum and endotoxin.

A study was done to determine if normal rabbits could be made tolerant to augmented pyrogenic action of endotoxin mixed with normal serum.

Five normal rabbits were given daily intravenous injections of 1.0 mcgm. of Shigella toxin which had been incubated for 30 minutes with 4.0 ml. of normal serum. Six additional animals were given daily intravenous injections of 1.0 mcgm. of toxin which had been incubated for 30 minutes with 4.0 ml. of 0.85 per cent NaCl. On day 21 the 2 groups of rabbits were reversed. Temperatures were measured on the twentieth and twenty-first days (*Fig. 7*). The animals given repeated injections of endotoxin-in-serum had higher fevers on the twentieth day than those given endotoxin-in-saline. Reversal of the groups on the following day resulted in higher fevers in animals given endotoxin-in-serum for the first time. The degree of tolerance of animals given repeated injections of endotoxin-in-serum was indistinguishable from that of rabbits injected daily with endotoxin-in-saline when either preparation was used as a challenge.

B. *Factors influencing the effect of tolerant rabbit serum upon the febrile response to endotoxin.*—Tolerant rabbit serum in contact with pyrogen fails to

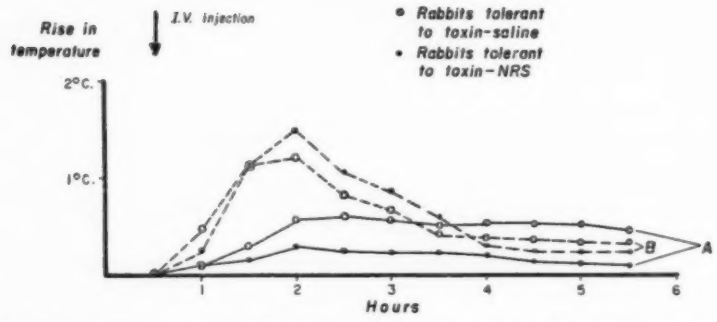

FIG. 7. Febrile response of rabbits tolerant to Shigella endotoxin-in-saline and endotoxin-in-normal rabbit serum. A. Injection of 1.0 mcgm. Shigella toxin incubated with 4.0 ml. saline for 30 minutes. B. Injection of 1.0 mcgm. Shigella toxin incubated with 4.0 ml. normal rabbit serum for 30 minutes.

enhance fever (4, 7). The following experiments were done to determine if the period of contact with toxin influences the apparent inability of this serum to influence the febrile response and if the properdin system plays a role in the lack of enhancement.

Two groups of 3 tolerant rabbits were used. Each of the animals in one group was injected intravenously with 1.0 mcgm. of Shigella endotoxin which had been incubated for 30 minutes with 4.0 ml. of serum from animals tolerant to Shigella toxin. The other group was given 1.0 mcgm. of toxin which had been incubated for 12 hours with 4.0 ml. of the same serum. On the following day the 2 groups were reversed and the experiment was repeated.

Serum from rabbits tolerant to homologous endotoxin did not augment fever irrespective of the time of incubation at 37°C.

The tolerant rabbit serum in the preceding study contained precipitins for the Shigella endotoxin. To exclude the specific antibody as a factor in the failure to enhance fever, the following experiment was done.

Three rabbits tolerant to 1.0 mcgm. of Shigella endotoxin were injected intravenously with 1.0 mcgm. of Shigella toxin which had been incubated for 30 minutes at 37°C. with 4 ml. of serum from rabbits tolerant to 10 mcgm. of P-35. A second group of 3 tolerant animals received 1.0 mcgm. of Shigella toxin which had been incubated for 12 hours at 37°C. with 4.0 ml. of the same tolerant serum. The experimental groups were reversed on the next day and the experiment was repeated.

The mean fever curves obtained were similar to those described in the preceding experiment, showing that serum from rabbits tolerant to the pyrogenic action of an endotoxin from a heterologous microörganism is unable to augment fever.

Landy and Pillemer (10) have shown that, under certain circumstances, inoculation of endotoxin into normal animals is followed by a transient rise in serum properdin levels and a parallel increase in resistance to Gram negative bacterial infection. The role of properdin in development of tolerance to fever following repeated inoculations of endotoxin was investigated in the following experiments.

Six normal rabbits were injected intravenously with 1.0 mcgm. of Shigella endotoxin daily for 14 days. Ten ml. blood was collected aseptically by cardiac puncture prior to injection of toxin on the first and fourteenth day. The serum was separated and stored promptly at −50°C. Properdin assays were performed on paired serum specimens from each animal and the sera were also analyzed for their effect on pyrogen fever in tolerant animals.

The serum collected after the development of tolerance failed to augment pyrogen fever although there was no change in properdin titers with tolerance (Table I). This suggests that serum properdin plays no role in this type of acquired resistance to endotoxin and that the failure of tolerant rabbit serum to augment endotoxin fever is not attributable to the properdin system.

Three rabbits tolerant to 1.0 mcgm. of Shigella endotoxin were injected intravenously with 1.0 mcgm. of Shigella toxin which had been incubated at 37°C. for 30 minutes with 4.0 ml. of serum from rabbits tolerant to 10 mcgm. of P-35. A second group of 3 tolerant animals was injected intravenously with 1.0 mcgm. of Shigella toxin which had been incubated at 37°C. for 30 minutes with 4.0 ml. of zymosan-treated serum from rabbits tolerant to 10 mcgm. of P-35. This experiment was repeated in 6 animals using serum from rabbits tolerant to 1 mcgm. of Shigella toxin, and a similar study was carried out with mixtures of endotoxin and tolerant serum which had been incubated at 37°C. for 16 hours.

TABLE I

Properdin Titers of Normal and Tolerant Rabbits

Rabbit	Units of properdin per ml. serum			
	Day 1		Day 21	
	Uncentrifuged	Centrifuged*	Uncentrifuged	Centrifuged
239	30	42	36	42
240	30	42	18	18
244	24	30	30	36
250	4	4	4	4
254	30	30	30	36
255	30	30	24	24

* Aliquots of serum centrifuged at 35,000 g. for 2 hours at 2°C. Properdin titers of rabbits 24 hr. before the first of daily I.V. injections of 1 mcgm. Shigella endotoxin, and 24 hours after the last inoculation.

Treatment of tolerant rabbit serum with zymosan did not alter its inability to enhance endotoxin fever, further evidence that properdin is not involved in the loss of this property.

Heating at 60°C. for 2 hours or storage at 4°C. for one month did not restore the ability of tolerant serum to augment the febrile response to endotoxin.

C. *Effect of passive transfer of normal serum and exchange transfusion of normal blood on the febrile response of tolerant rabbits to endotoxin.*—To determine if tolerant rabbits can be made more responsive to the pyrogenic action of endotoxin by the administration of normal serum or blood, the following experiments were performed.

Six tolerant rabbits were given intravenously a total of 70 or 80 ml. of normal rabbit serum in 3 separate injections over a period of 18 hours. The animals were given immediately 1.0 mcgm. of Shigella toxin in 0.85 per cent NaCl and febrile responses were recorded. There was no change in time of onset or height of the fevers in these animals after injection of this amount of normal serum.

Four tolerant rabbits were transfused with 120 to 180 ml. of normal rabbit blood. Pooled blood for transfusion was collected from unanesthetized normal donor animals into Fenwal plastic bags after passage over a sterile ion exchange column. Four ml. aliquots of blood were shown to augment the pyrogenic action of 1.0 mcgm. of Shigella endotoxin when incubated for 30 minutes *in vitro* and injected into tolerant test animals. Replacement transfusions were carried out by bleeding the anesthetized (sodium pentobarbital) recipient animal by cardiac puncture during infusion of an equal quantity of the normal blood into an ear vein over a period of 3–5 minutes. Eighteen hours later, the transfused animals were challenged with 1.0 mcgm. of Shigella endotoxin-in-saline. There was no change in the febrile responses to endotoxin of the rabbits after transfusion.

DISCUSSION

The *inhibition of endotoxin fever* which occurs after prolonged contact of endotoxin with normal serum can be correlated with properdin activity. This is indicated by the lability to heat and storage of this property and its removal by zymosan under appropriate conditions. Hegemann (6) has suggested that the inhibitory effect of normal serum is the result of enzymatic hydrolysis of the endotoxin. That other factors may play a role is suggested by the recent studies of Stauch and Johnson (11) and Ho and Kass (12) which indicate that normal serum can alter antigenicity and protect against the lethal action of crude endotoxin in the absence of properdin.

Augmentation of endotoxin fever by normal serum is not correlated with properdin activity. This is indicated by stability of this property to heat and

storage, and the failure of treatment with zymosan to alter it. Farr and others (1–4, 7) have shown augmentation of fever when normal serum and endotoxin are in contact for less than 5 minutes at 4°C. or 37°C. Furthermore, contact of serum and toxin at 4°C. for many hours results only in augmentation of fever, although prolonged contact at 37°C. inhibits the febrile reaction. The facts that increasing volumes of serum produce increasing enhancement of the febrile response to a fixed dose of endotoxin and that the reaction is independent of temperature suggest that enhancement of endotoxin by serum does not involve enzymatic action. The factor, or property, of serum responsible for this influence on endotoxin fever remains unidentified.

Westphal (13) has shown that the lipid fraction of the protein-lipo-polysaccharide endotoxin complex is responsible for toxicity. In addition, it has been found that cholesterol, lecithin and normal serum interfere with certain serological activities of endotoxin (14). The possibility that cholesterol or lecithin might also alter the toxic action of endotoxin was suggested (14) but has not been demonstrated.

It has been demonstrated that serum from rabbits tolerant to bacterial pyrogen does not augment the febrile response (4, 7). Inoculation of endotoxin mixed with normal serum into tolerant rabbits, however, enhances fever. The fact that normal serum containing properdin does not augment pyrogen fever after prolonged contact at 37°C. suggested that properdin might be responsible for the absence of an augmentation effect of tolerant serum. The properdin titer of tolerant rabbit serum, however, does not differ from that of normal serum, and removal of the properdin with zymosan does not restore the augmenting activity to tolerant serum. It seems probable, therefore, that tolerance is accompanied by *depletion* or *inactivation* of a serum factor responsible for altering the pyrogenic effect of endotoxin.

It is difficult to understand why infusion of a large volume of normal serum or exchange-transfusion does not reconstitute the normal reactivity of tolerant rabbits, if the augmenting effect of serum plays any important *in vivo* role in the febrile response to endotoxin. The effect of normal serum on pyrogen fever produced by incubation *in vitro*, may well have no importance in the biological action of endotoxin. Farr (7) demonstrated that addition of tolerant rabbit serum to normal serum prevented the normal serum from augmenting fever, suggesting that tolerant serum contains an inhibitor of the pyrogenic action of endotoxin. It is conceivable that such an inhibitor is responsible for the failure of infusion of normal blood or serum to restore reactivity. It is interesting, however, that infusion of tolerant serum into normal animals does not appreciably alter the febrile response to endotoxin (7).

Rabbits continue to react with an augmented febrile response when given repeated injections of endotoxin that has been incubated for a short time with normal serum. This observation, coupled with the findings already discussed,

is important in an interpretation of the characteristics of "endogenous pyrogen" described by Atkins and Wood (15). These investigators found that the blood of rabbits inoculated intravenously with endotoxin contained two pyrogenic substances with different biological properties. During the first few minutes after injection of endotoxin, the pyrogen in the animals' blood produces febrile reactions in normal animals typical of the inoculated toxin, but produces little or no fever in tolerant rabbits. This initial circulating pyrogen is replaced by a second pyrogenic substance that produces fever in both normal and tolerant rabbits. Petersdorf and Bennett (16) have shown that this "endogenous pyrogen" is heat labile and does not induce tolerance. Furthermore, in the presence of normal serum, the ability of endotoxin to produce fever becomes similarly susceptible to heat. "Endogenous pyrogen" may be a distinct substance different from the injected endotoxin, but the findings presented here, show that the properties of endotoxin in serum and "endogenous pyrogen" are not clearly separable. The possibility remains that "endogenous pyrogen" is endotoxin altered by contact with serum and not a substance newly derived from injured cells. Braude, Carey and Zalesky (17) have studied the distribution of isotopically labelled endotoxin in blood, lung, and liver after intravenous inoculation, and have shown that radioactivity disappears rapidly from the circulation and does not reappear. This finding, in addition to the analysis of Atkins and Wood, therefore, leaves unsettled the question of whether "endogenous pyrogen" is a product of tissue injury or endotoxin in an altered form.

SUMMARY

The studies reported differentiate clearly two separate effects of normal serum upon the pyrogenic activity of bacterial endotoxin. Contact of serum and endotoxin *in vitro* for periods up to 30 minutes at 37°C. or for many hours at 4°C. results in augmentation of pyrogen fever. The extent of this effect is partially dependent upon the volume of serum used and the ability of serum to enhance the febrile response to endotoxin is retained after heating at 60°C. for 30 minutes or storage for one month at 4°C. The serum of animals that are "tolerant" to endotoxin does not augment pyrogen fever.

Contact of normal serum and endotoxin for 8–12 hours at 37°C. results in a decreased pyrogenic activity. This ability of serum to inhibit endotoxin fever is destroyed by removal of properdin with zymosan, by heating at 60°C., or by storage at 4°C. for one month.

The serum properdin levels of "tolerant" animals are normal, indicating that the failure of their serum to augment endotoxin fever is not related to this system. The ability of tolerant rabbits to react to endotoxin with fever was not restored by transfusion of large amounts of normal serum or blood. There is no evidence that the inability of serum from tolerant animals to en-

hance the activity of endotoxin is involved in their reduced ability to respond to injected endotoxin with fever.

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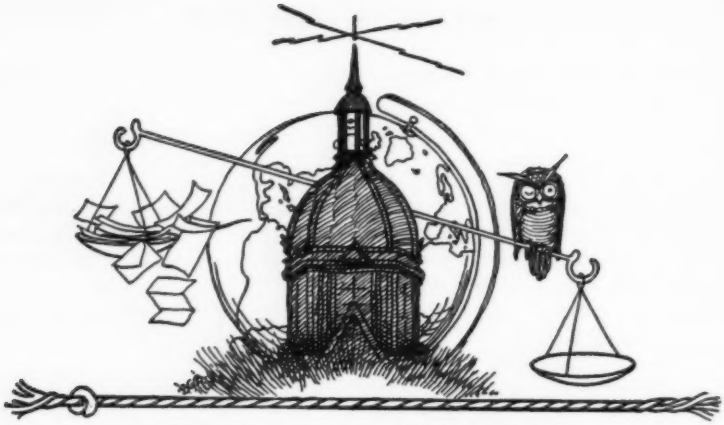

BOOK REVIEWS

Systemic Arterial Embolism. Pathogenesis and Prophylaxis. By JOHN MARTIN ASKEY. 157 pp., $5.75. Grune & Stratton, New York, N. Y.

This small volume (157 pages) is concerned with the current status of knowledge in the field of embolism of the systemic arterial tree. In general the areas touched on are handled fairly objectively with review of some of the published data on both sides of moot points and fairly reasonable conclusions drawn. In certain instances, however, there are glaring omissions and in others there is a very casual statistical attitude which reduces the discussion to an expression of clinical opinion.

As examples of points omitted may be cited the absence of any reference to the role of heparin, and of any discussion of the therapeutic measures (other than embolectomy) employed in the management of an individual embolic episode. Where these very germane subjects are skirted, a long chapter on the differential diagnosis of heart disease has been included, gratis, as it were. Examples of the casual statistical attitude are found in such undocumented statements and headings as "Estimated chance of . . . death from eventual embolism . . ." (p. 43) and "Although the available evidence is incomplete and inaccurate, still it indicates that . . ." (p. 16).

In addition to this occasional lack of objectivity there are one or two important typographical errors which add to the impression that this monograph needed a thorough going over by a kindly but critical reviewer before its printing. I say kindly because despite its faults, there is enough good material here to represent the framework of a good piece of work had it been approached more critically and in more detail. Not all of its contents are acceptable in present form, though some valuable points are made and the reference table is good.

ROBERT E. MASON

Physical Examination in Health and Disease, 2nd edition. By RUDOLPH H. KAMPMEIER. 774 pp., $9.50. The F. A. Davis Co., Philadelphia, Pa.

This is a revised and enlarged edition of a successful textbook devoted to physical diagnosis. It is written for the second year medical student. The introduction and first chapter which give the historical development of physical diagnosis, and comments on the "art" of history taking are particularly well written and informative and present penetrating ideas valuable not only to medical students but also to physicians.

The whole subject is divided into regional anatomical units to which a chapter presenting normal physical findings and a second chapter presenting abnormal findings are devoted. The dichotomy between normal and abnormal is thereby well established, perhaps even too much so!

The book is extremely well illustrated. To be particularly praised is the practice of showing examples of early, or minimal lesions as well as examples of advanced lesions. All too often in textbooks of this sort only examples of the most advanced lesions are shown; such clinical situations are really seldom encountered nowadays. The presentation of examples of minimal lesions also introduces to the second year student the concept of preventive medicine in a very ingenious and subtle way. The book is recommended to student and physician alike.

JOHN COLLINS HARVEY

Optics. The Science of Vision. By VASCO RONCHI. Translated by Edward Rosen. 360 pp., $10.00. New York University Press, New York, N. Y.

The author of this book is Director of the National Institute of Optics, Arcetri, Italy. In its subject matter and treatment it lies midway between the conventional textbook on optics, which might be used in a course in physics, and that written specifically for the ophthalmologist or the optometrist. As supplementary reading it could be of interest to both groups in giving a fresh point of view on an already familiar subject.

The first portion of the book discusses the definition and scope of optics and presents interesting material on its historical development. Following this are three chapters on vision by means of optical systems, acuity of vision and the optical image. Here and there the ophthalmologist can find occasional items of information applicable to his daily work. For example, those who have had experience in prescribing magnifiers for the partially blind will recognize the importance of the following comments on page 283:

> "Nobody has ever thought that many instruments showing defects intolerable for observers with very acute sight may be ideal for observers with inferior vision. If particular consideration were given to these observers, who are far from rare, rules might be discovered for designing and manufacturing cheaper and more useful instruments. For instance, observers with poor vision probably would derive greater benefit from instruments with extreme magnification even though that entailed a greater distortion of the field".

It is unlikely that this book will be of interest to very many ophthalmologists. They may, however, benefit from it indirectly if it serves to remind the physicist that the characteristics of the human observer play an important role in the subject of optics.

LOUISE L. SLOAN

Clinical Physiology. The Functional Pathology of Disease. Edited by ARTHUR GROLLMAN. 854 pp., $12.50. McGraw-Hill Book Co., New York, N. Y.

The twenty-six distinguished contributors to this volume probably insure its worthwhileness. Like Sodeman's excellent *Pathologic Physiology* this work is designed to be intermediate between a textbook of physiology or of biochemistry and a textbook of medicine. Yet it is neither of these and perhaps it is best to regard it as a series of monographs expressing the views and experiences of the individual contributor.

There are several excellent chapters on respiratory physiology in health and disease by Richards and Cournand. There are three chapters by the late J. P. Peters on fundamentals of carbohydrate, fat and protein metabolism which essentially represent a modernization of his last work in his familiar textbook. Chapers on electrolyte physiology and renal function are contributed largely by former associates of Dr. Peters whose views are thoroughly familiar by now. Frank Engle's chapter on the adenophypophysis is an inspired synthesis

of the most important information available in this broad and complicated area and represents a unique and valuable contribution. Bean's chapter on the vitamins is a delight to read.

On the other hand there are a number of inadequacies. Some of the chapters are so sketchy that one wonders if they are included simply for the sake of giving completeness to the index. The introductory chapter on energetics and metabolism could have been written years ago.

Here and there throughout the book one finds minor errors in statement. For example, it is incorrectly said that there is turbulent flow through most of the arterial tree; that McArdle's syndrome is the result of lack of 1,3-diphosphoglyceraldehyde dehydrogenase (McArdle made no such claim; he simply pointed out the similarity between observations in his patient and the effects of iodo acetate poisoning *in vitro* and suggested that the two might have a common mechanism.); that pulmonary edema occurs in patients with acute renal failure as a result of myocardial damage. The statement that there is an effect of adrenal insufficiency on muscle function with the implication that there is a direct effect of adrenal lack on muscle with consequent weakness ought to be supplemented by reference to those papers that show quite well that much if not all of the weakness is secondary to impoverished peripheral circulation. It is also past time for the words renal clearance to be drummed out of the literature with the reservation that they be retained for small print in historical sections. Modern treatments of renal function ought to avoid the confusion produced in students' minds by the mythical concept of clearance and should get to the heart of the matter by clearly discussing rates of filtration, reabsorption and secretion. In the main these are unimportant reservations. The large bulk of contributions are lucid expositions of physiological and chemical lesions underlying disease.

KENNETH L. ZIERLER

Hypothalamic-Hypophysial Interrelationships. A Symposium. Edited by W. S. FIELDS, R. GUILLEMIN, AND C. A. CARTON. 156 pp., $4.75. Charles C Thomas, Springfield, Ill.

Neuroendocrinology is such a rapidly expanding field that the proceedings of a symposium such as this one are dated before they reach the reviewer. The dating usually becomes more serious after the monograph has been kept in the reviewer's briefcase for a few months.

There were eight papers presented at this symposium which was held in Houston two years ago. Of these eight, two were purely anatomical. The one by J. D. Green reviews the literature on brain-pituitary connections. It led off the symposium and must have served as a firm anatomical framework for the papers and discussions which followed. The paper by Gloor is an excellent treatment of forebrain connections with the hypothalamus. Although the present state of our knowledge allows few conclusions regarding the physiological meaning of such connections, Gloor indulged in some conservative and interesting speculations.

The next paper was by Professor G. W. Harris. He contributed a timely review of the literature which must have brought the members of the symposium up to date on the subject of central nervous control of the anterior pituitary.

Walther Hild's description of the behavior of the neurosecretory substance under various environmental and experimental conditions is an exciting one. It whets one's appetite for seeing some of his data, none of which are presented in the monograph.

The *in vitro* studies of Guillemin which have drawn so much attention in both the lay and scientific presses during the past two years are described in some detail. Although some of the data are reproduced in the book, the reader will probably wish for more if he is statistically inclined.

Very interesting data from their studies of the endocrine effects of brain stimulation in humans were presented by Pool and his collaborators at Columbia. The experiments are

described and the data presented in each case in sufficient detail for the reader to decide for himself upon the merits of the study and the conclusions drawn from it.

The final paper in the symposium dealt with hypothalmico-hypophysial dysfunction in man. The first one-third of the presentation consisted of a review of clinical tests for pituitary function. The remainder was taken up by presentation of findings on one patient. This constitutes eight pages of data on pituitary function in a patient who had a history which included the following entries (listed chronologically, here): paranoid schizophrenia, polydipsia and polyuria of several years duration, X-ray treatment for a suspected pituitary tumor, symptoms of an expanding intracranial lesion, surgery for removal of a large chromophobe adenoma which had invaded the hypothalamus, and a partial frontal lobectomy.

Since he is far removed from clinical endocrinology, your reviewer does not quite see how collection and publication of data from such a complicated preparation contributes to our understanding of hypothalamico-hypophysial interrelations.

J. W. Woods

Ultramicro Methods for Clinical Laboratories. By Edwin M. Knights, Jr., Roderick P. MacDonald and Jaan Ploompuu. 128 pp., $4.75. Grune and Stratton, New York, N. Y.

There is an old story among chemists about micro-analysis in which, as the sample gets smaller and smaller, the analytical error gets less and less until finally one can dispense with the sample completely and have no error at all. Ultramicro methods for clinical laboratories make a lot of sense. As the number of determinations per patient increases, exsanguination by chemistry becomes a problem. In a large hospital laboratory the saving in quantity of reagents through microanalysis is a genuine economy, and accuracy is at least as good as with macro methods.

This slim volume is a practical compendium of methods in use in one hospital. It is not a complete summary of available methods, but it does describe a number of the most commonly used tests, each done on 0.01 to 0.03 ml. of serum, permitting a fairly complete survey to be done on 0.2 to 0.3 ml. of blood obtained by pricking a finger.

The presentation is cook-book style, quite clear and should be a useful starting place for those interested in clinical chemistry.

Kenneth L. Zierler

BOOKS RECEIVED FOR REVIEW

Anesthesia and Otolaryngology. By Donald F. Proctor. 267 pp., $7.00. The Williams & Wilkins Co., Baltimore, Md.

Animals Parasitic in Man. By Geoffrey Lapage. 321 pp., 95 cents. Penguin Books, Inc., Baltimore, Md.

Clinical Orthopaedics No. 9. Edited by Anthony F. De Palma. 353 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Current Surgical Management. Edited by John H. Mulholland, Edwin H. Ellison and Stanley R. Friesen. 494 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

From Sterility to Fertility. By Elliot E. Philipp. 120 pp., $4.75. Philosophical Library, New York, N. Y.

Histology, 3rd edition. By Arthur Worth Ham. 894 pp., $11.00. J. B. Lippincott Co., Philadelphia, Pa.

Methodology of the Study of Ageing. Ciba Foundation Colloquia on Ageing, Vol. 3. Edited by

G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR. 202 pp., $6.50. Little, Brown and Co., Boston, Mass.

One Surgeon's Practice. By FREDERICK CHRISTOPHER. 151 pp., $4.00. W. B. Saunders Co., Philadelphia, Pa.

The Ordeal of Gilbert Pinfold. By EVELYN WAUGH. 232 pp., $3.75. Little, Brown & Co., Boston, Mass.

Peripheral Nerve Regeneration. VA Medical Monograph. Edited by BARNES WOODHALL AND GILBERT W. BEEBE. 671 pp. For sale by the Superintendant of Documents, U. S. Government Printing Office, Washington, D. C.

The Psychologic Study of Man. By JOHN MONEY. 216 pp., $4.75. Charles C Thomas, Springfield, Illinois.